Dietary diversity and resource continuity improve fitness and alter flight in a native coccinellid predator, *Hippodamia convergens* (Coleoptera: Coccinellidae).

by

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Abstract

Conservation biological control methods seek to support healthy communities of beneficial predators that can provide predation and pest control services in the agroecosystem. Manipulation of resource availability through land management practices can provide critical support to the survival and ecosystem services of beneficial insect predators. Thus, identifying necessary resource types and ideal arrangement and timing of these resources can improve the efficacy of this land management and improve predation services. *Hippodamia convergens* Guerin-Meneville is an important generalist insect predator that provides predation services in economically important agricultural systems, especially upon aphids. This species exploits both prey and plant-derived resource and moves among crops throughout the growing season in response to, and in anticipation of, pest populations. Dispersal flight is necessary to discover widely distributed and ephemeral resources, but movement can be metabolically costly and reduce energetic capital for later reproduction and movement expenditure. Discontinuity of resources resulting from spatially and temporally scattered patches influences *H. convergens* beetles by increasing the need for dispersal. This thesis explored the effects of diet composition and resource continuity on *H. convergens* development, reproduction and flight capacity, while simultaneously investigating life history tradeoffs between reproduction and dispersal by flight. The effects of diet composition on development, reproductive performance and flight were explored by varying feeding conditions. A monotypic, prey-only diet yielded faster development, and larger adults, but delayed oviposition and reduced fecundity relative to a diet comprised of diverse prey and plant resources. Plant resource availability in the diet was found to hasten oviposition, increasing the number of oviposition days and overall fecundity. Larger adults

resulting from greater resource access as larvae delayed oviposition longer and laid fewer clutches, though fecundity was not affected. Potential reproductive effects of diverse resource access include increased population growth, either due to speeding oviposition start times, or through directly increasing egg numbers. Results of flight assays with plant resource diets showed marginal interactions with adult body size, but neither diet composition nor adult size had significant effects on flight distance. Post-reproductive flights were significantly reduced relative to pre-reproductive flights in the same treatment. Thus even though diet treatments were not shown to directly influence flight capacity under our short observation periods, we did see apparent costs of reproduction on later flight. Resource access continuity effects were examined by varying periods of food access and food amount and measuring reproduction and movement capacity in adult beetles. Discontinuous availability of diet components was shown to delay onset of oviposition, reduce individual reproductive days and fecundity, though flight performance was only influenced indirectly through reproductive effects. Thus continuity of resource access can improve biological control services through improvements to population growth rates through faster oviposition and greater egg production. Taken together, these results illustrate important potential benefits of increased plant and insect diversity, along with increased resource continuity to the success and sustainability of biological control programs in the agroecosystem. The results of these studies increase our understanding of the nutritional needs of these important predators and could help to shape future land management strategies to improve biological control. Future work should examine the specifics of flight behavior in conjunction with metabolic activity and examine responses to other prey species and supplemental resources for potential effects on *H. convergens* fitness and life history.

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Acknowledgements

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Chapter 1 - General Introduction

1.1 | INTRODUCTION

Agricultural intensification dominates much of the American High Plains, (Nickerson et al 2007) which is characterized by widespread monocultures, and a fixed set of crop rotations within and among years. Though this system favors efficiency of planting and harvesting, the spatial (Kennedy 2016) and temporal (Iuliano and Gratton 2020) disturbances resulting from agricultural practices can create significant challenges to beneficial insects and the ecological services they provide in the agroecosystem (Bianchi et al 2006, Chaplin-Kramer et al 2011). Cultural practices such as tillage and chemical control of weeds and insect pests can increase spatial patchiness of resources and contribute to high levels of disturbance, harming predatory insects within the agroecosystem (tillage: House and Parmelee 1985, Alyokhin et al. 2020, Rowen et al. 2020, pesticides: Relyea and Edwards 2010, Jonsson et al. 2012). The negative effects of spatial disturbance on insect biodiversity and ecosystem function are well documented (Bianchi et al 2006, Chaplin-Kramer et al 2011, Tscharntke et al 2012) and include decreased species abundance, richness and evenness, including that of predators and parasitoids (Corcos et al 2019, Agra et al 2021) along with decreased services (Thom and Seidl 2015) including predation (Schellhorn et al 2015) and pollination(Williams et al 2012, Riedinger et al. 2014, Malfi et al. 2020). As a complement to spatial continuity more recent work has examined the effects of temporal disturbance and resource discontinuity on conservation biological control (CBC) (Solbreck 1995, Angeler 2016, Welch and Harwood 2014, Schellhorn et al., 2015, Haan et al., 2020, Spiesman et al., 2020). Understanding how temporal resource dynamics may act in concert with, and in counterpoint to, spatial dynamics can be important to crafting effective land

management strategies that cooperate with human needs for agricultural lands while still ensuring the maximum possible support to important ecosystem services such as predation.

Generalist insect predators are important to integrated pest management (IPM) in agriculture as their wide dietary niche reduces starvation vulnerability and increases the range of potential pests that one predator can control (McKinney 1997, Biesmeijer et al 2006, Colles 2009). Lady beetles (Coleoptera: Coccinellidae) are especially important to biological control in the American Midwest and are present in nearly every agricultural system worldwide (Rice and Wilde 1988, Nechols and Harvey 1998, Colares et al 2015).The majority of coccinellids are predatory, but many species also exploit plant resources and may depend on plant-derived resources such as pollen, nectar, and leaf tissue (Jalali et al 2009, Lundgren 2009, He and Sigsgaard 2019, including in *Hippodamia convergens,* Mercer et al 2020) for diet supplementation. This trophic flexibility can benefit these beetles as it can aid in survival when prey resources are scarce (Hodek 1996, Hodek and Evans 2012, McKinney 1997). In spite of the benefits dietary generalism can afford, the spatial and temporal disturbances inherent in large-scale farming operations may still hamper population growth and dispersal capacity in *H. convergens.* On the High Plains many species of coccinellids reproduce in a different crop field than their natal patch (Bastola et al 2016), often switching diets in accordance with the availability of prey and plant resources (Michaud 2018). Using stable isotopes, Prasifka et al. (2004) showed that *H. convergens* moves between fields in response to differential prey availability, emigrating from early season crops to later season crops even before they have developed large populations of aphid prey. This would suggest that *H. convergens* dispersal behavior has evolved in response to resource variability on landscape scales.

In discontinuous and fragmented landscapes with resource poor matrices between resource rich patches, dispersal becomes critical to survival for many insect predators. The ability to track prey populations as they move from field to field as crops develop can determine predator population survival and the effectiveness of the biological control services they provide(Wissinger 1997, Landis 2000). Mobile predators readily move between habitats when resources become depleted and alternative resources are nearby (Wissinger 1997, Landis 2000), this high mobility may be a key determinant of coccinellid fitness, as well as their success in agricultural environments (Wissinger 1997, Xiao 2017). However flight is energetically costly and excessive flight distance or increased flight necessity may become metabolically burdensome on predators when food supplies become limited or disrupted.

1.1.1 Tradeoffs

Mobility around the landscape is important for survival in a spatially and temporally discontinuous agricultural landscapes, and it can also impact *H. convergens* fitness through interactions with reproduction (Wissinger, 1997; Landis et al., 2000; Prasifka 2004). Movement capacity is integral to effective resource utilization, and as such is often a critical concern for conservation and biodiversity support (Nathan et al. 2008, Jeltsch et al. 2013). For example, flight can increase survival through improved access to resources, thus increasing reproductive opportunities and fitness (Allen and Singh, 2016; Doherty and Driscoll, 2018). By contrast, increased flight will also consume energetic capital, which lowers energetic reserves that could otherwise fuel future reproduction, while also increasing exposure to the direct mortality risks of movement (O'Rourke and Peterson 2017). Thus, flight can increase fitness by extending survival and providing access to high quality resources, but greater flight effort can also become costly

and potentially reduce fitness through consumption of energetic capital and greater exposure to risk. The spatial and temporal distribution of resources in an agricultural landscape will affect this trade-off via diet composition or resource continuity effects, thus influencing *H. convergens* predatory performance and population dynamics. At a larger scale, movement capacity in resource patches with high spatial and temporal resource variability can alter the population growth rate and persistence (Russell and Ruffino 2012). Expanded individual movement capacity can increase access to far ranging resource rich locations and, especially in highly variable landscapes can provide a means of disturbance escape.

1.1.2 Thesis aims

Overall I explored how diet influenced the development, reproduction and movement of *Hippodamia convergens*. My research objectives explored how resource composition and continuity of access altered *H. convergens'* behavior and reproduction to better understand how multiple avenues of resource influence can act alone or in concert to alter their ecological performance. Chapter 2 of this thesis addressed diverse versus monotypic diet composition to determine the amount and direction of influence on larval development rate, final adult body size, and reproductive success. This examination of compositional diet diversity effects on fitness is expanded in Chapter 3 to determine the importance of plant resources as a dietary component of these insect predators. In Chapter 3, I manipulated larval food access to create two adult body size classes that reflected natal patch quality, and then focused on adult diet effects. I also examined flight distance capacity both before and after an 18-d reproductive period to ascertain dietary effects on dispersal behavior, and to ascertain any energetic trade-offs between reproduction and flight. In Chapter 4, I examined effects of resource access continuity in lieu of

resource composition, varying resource amount to control for the natural resource amount variation access discontinuity creates and measured direct effects of food quantity and continuity of access on reproduction and flight. Flight was also used here to investigate the presence and magnitude of energetic trade-offs between dispersal and reproduction. Finally in Chapter 5, I discuss general conclusions of chapters 2-4 in relation to the fitness success and biological control services of this species on a spatially and temporally discontinuous and compositionally variable agricultural landscape along with some ecological implications of this work.

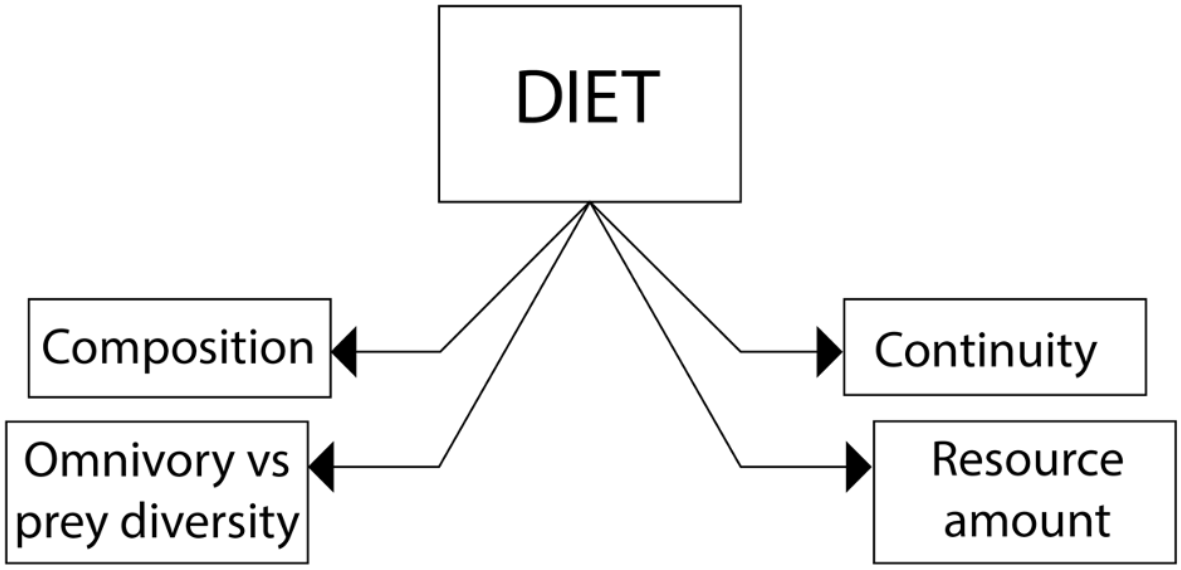

Figure 1 Conceptual map of the thesis project. I explored potential effects of resource variability on *H. convergens*, an important biological control agent. My research objectives investigated resource composition, continuity, and amount effects to understand how diet factors may influence important ecological performance metrics such as flight and reproduction.

1.2 | STUDY SYSTEM

Hippodamia convergens Guerin-Meneville is a native insect predator (Giorgi et al. 2009,Vargas 2013) that relies on aphids for reproduction but also exploits and relies on diverse prey and plant derived food components for survival and reproduction (Sloggett and Majerus 2000, Michaud and Qureshi 2006, Michaud 2018, Mercer 2020). This species provides predation services in agriculture throughout the growing season and has a flexible feeding strategy which has proven adaptable to the low plant diversity, common disturbances, and other challenges of the agricultural environment (Sloggett and Majerus 2000). For example, omnivory, especially the consumption of plant-derived resources is important to both the survival and reproduction of *H. convergens* lady beetles. The consumption of pollen, nectar and tender foliage may supply both hydration and nutrients to aphidophagous lady beetles (Hodek 1996, Hodek and Evans 2012) while also providing a source of phytosterols that can be critical to fertility in the diet of omnivorous predators (Ugine et al. 2019). Carnivory on the other hand, especially intraguild predation, can be a critical source of nitrogen in the diet of predatory arthropods (Denno and Fagen 2003, Fagan and Denno 2004, Raubenheimer et al. 2007). In addition to supplying important trace nutrients, omnivory by *H. convergens* may also allow these insects to optimize their macronutrient intake (Jensen et al. 2012), as carnivorous and herbivorous diets often differ in macronutrient ratios (Raubenhiemer and Jones 2006, Raubenhiemer et al. 2007). Flexibility in diet composition is therefore important to maximize reproductive rates and support the survival of omnivorous predators that are key contributors to biological control of pests. *Hippodamia* convergens' dispersal capacity (Hagen 1962, Bianchi et al. 2009, Jeffries et al. 2013), and its omnivorous habits (Michaud and Qureshi 2006, Mercer et al. 2020) facilitates its movement between crop fields and the persistence of its population in agroecosystems when food resources are low (Prasifka 2004). Though prior work investigating the dispersal capacity of this species is

relatively scarce, Rankin and Rankin (1980) observed flight times at an upper range of 2 hours, with mean flight times around 36.5 minutes, and documented flight altitudes between 1000 and 1650 meters (Jeffries et al. 2013). Flight periods chosen for these thesis experiments exceeded both the average times and the reported upper range times to provide adequate information about the flight behavior of this species. However, flight times observed in this thesis often exceeded these averages. Environmental temperature has been shown to influence flight behavior in *H. convergens* (Elliot 2000) as it does in most insects (Taylor 1963). Additional factors such as time of day, food consumption, and ovary development have also been shown to influence flight propensity and performance (Davis and Kirkland, 1982). However, more information is needed to investigate movement capacity of *H. convergens* to understand dispersal motivations and total flight capacity. Emigration behavior has long been noted in *H. convergens* as a barrier to their effective use in augmentative biological control (Davis and Kirkland, 1982), as these insects are unlikely to remain where they are applied even when prey in that region is plentiful. Total flight capacity, on the other hand, has broader ecological implications as this can affect population dynamics, resource access, migration, and foraging among other ecologically significant behaviors (Rankin and Burchsted 1992).

1.3 | FLIGHT MILL

1.3.1 Rationale

Movement capacity is an important component of insect behavior with consequences for both individual fitness and population dynamics. Ecological performance can show influence from distance capacity, speed, dispersal and migration ability, along with simply foraging. In

agroecosystems especially, greater movement ability can improve a species or population's resilience to disturbance and allow access to resources that are spatially distant and ephemeral in time (Sorribas et al. 2016). For many insects and especially insect predators, understanding individual movement often requires accurately and effectively measuring flight capacity. Flight behavior of individual insects can be influenced by many intrinsic and extrinsic factors including perceptive ability (Gotz 1968, Craig 1986), metabolism (Kammer and Heinrich 1978, Candy et al. 1995), life stage and age (Johnson 1960, 1966), temperature (Davis and Kirkland 1982), wind speed, and precipitation (Jeffries et al. 2013). The study of insect flight presents many challenges in the field due to the uncertainty of mark-recapture methods (Macleod 1958, Marker and Wineriter 1981) and prohibitive weight and challenges posed by miniaturization of remote tracking technologies (Fry et al. 2000, Noldus et al. 2002, Tahir and Brooker 2015). For these reasons among others, tethered insect flight has been used effectively to record and study flight duration, distance, and speed under controlled conditions (Naranjo 2018).

The simplest form of tethered flight studies uses static tethered flight, but this method has several significant limitations. Static flight testing consists of attachment to a static, suspended anchor point, often including a directed airstream to stimulate flight in the suspended insect (Davis 1986). Though this method requires the least equipment affords a quick set up and permits observation of wing beat frequency and aerodynamics, it does not allow accurate measurement of distance or speed (Naranjo 2018). Perhaps most importantly for the researcher, this method requires direct visual observation for data collection and only one insect's flight capacity can be recorded at a time. This substantially increases the number of observation hours necessary to collect a statistically meaningful amount of data and makes this method of flight data collection prohibitively time and work intensive.

Alternatively, the most sophisticated method of flight data collection is free flight data collection, which uses transparent flight chambers where unrestrained movement is monitored and recorded, either directly by a researcher or automatically via video and tracking software (Grace and Shipp 1988, Attisano 2015). This type of data collection allows for more natural flight patterns and can record distance, speed and interval of flight with the additional option of recording flight direction. However, again, the number of direct observation hours can be an obstacle for students on short timelines, and the sophisticated equipment required for video and movement tracking in flight is often inaccessible to newly established labs with a relative dearth of technical assistance and equipment. Even in labs with established flight mills the cost of maintaining the equipment can be burdensome.

Tethered flight mills can provide a happy medium between these two methodological extremes (Attisano 2015). Rotary mills allow for directed flight with no need for airstreams or sophisticated camera tracking, and they mills can be rigged to collect data automatically, eliminating the need for direct observation. Therefore, the use of tethered rotary flight mills splits the difference between the low cost – though time and labor intensive – process of fixed flight data collection and high cost – though more accurate and precise – options to record insect flight capacity.

1.3.2 Equipment

Insect flight represents a rich opportunity for biological and ecological research. However the time investment and scale of operations necessary to produce statistically relevant data can present an obstacle for successful completion of these studies. This has long been a challenge for small labs and students especially as these groups are often the most time and resource limited.

The price of flight mill apparatuses can be prohibitive and what instructions exist for bootstrapping original equipment can be intimidating for individuals who lack prior experience with electronics or programming. Technology exists however, to make an inexpensive, simple large-scale flight mill. For this thesis project I adapted the design originally published by Attisano et al (2015) with more affordable and accessible electronic components and additional 3D printed components that can be added to plexiglass junctures to stabilize the structure (Figure 2). I used magnetic attachment to connect insects to the mill apparatus and infrared sensors to record the speed, distance, and activity interval for individuals. The mill complexes required construction of plexiglass frames (2 mill x 4 mill design), electronic circuitry and wiring, and Arduino coding. This setup allowed testing of large numbers of individuals as each mill unit comprises 8 individual mills that can be run simultaneously with automated data collection. The entire construction is relatively small, and two such mill units fit in a standard growth chamber together. The design alterations reduce the total cost of each mill unit and aid in the stabilization and adaptability of the entire structure.

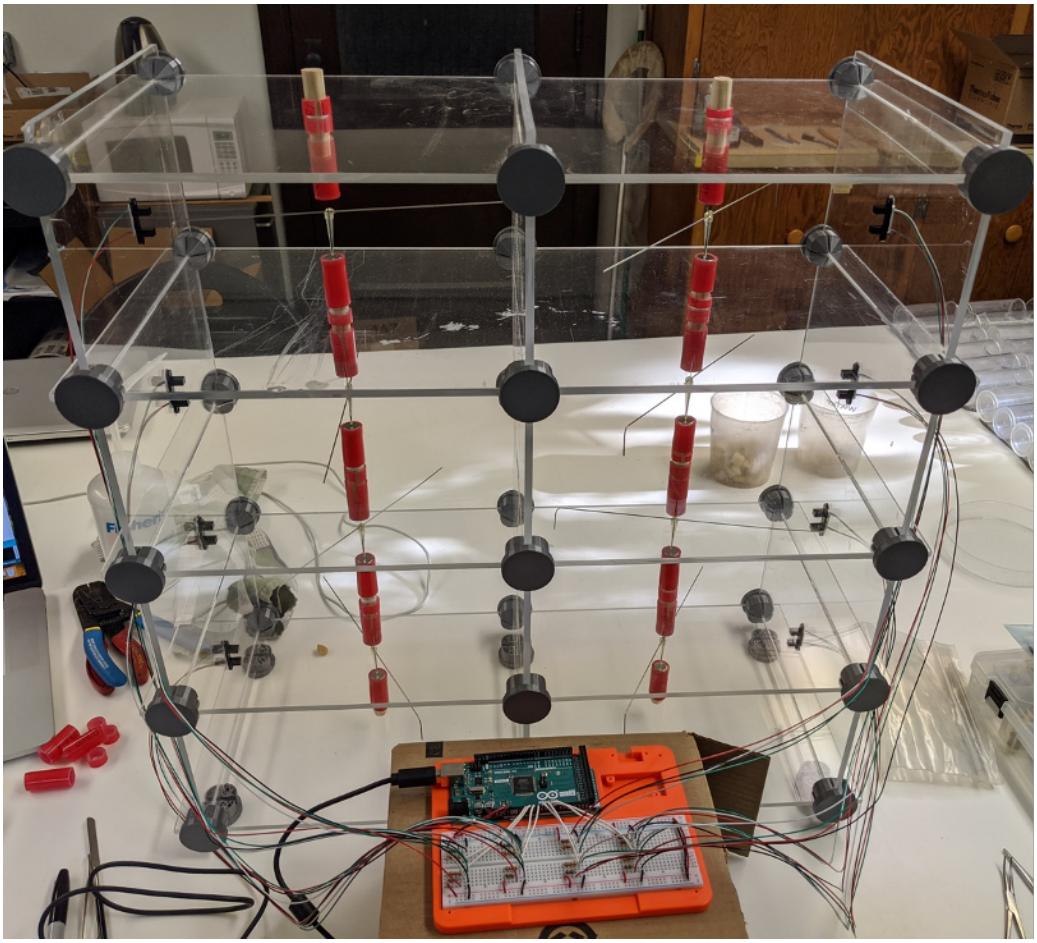

Figure 2 Tethered insect flight mill adapted from Attisano et al. 2015, 2 mill columns x 4 mill rows. Microcontroller and breadboard fitted into a 3D printed tray (orange) with stabilization pegs (charcoal) added at each juncture between vertical and horizontal plexiglass pieces.

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Chapter 2 - The Benefits of Omnivory for Reproductive and Life History in a Specialized Aphid Predator, Hippodamia convergens (Coleoptera: Coccinellidae).

2.1 | ABSTRACT

The convergent lady beetle, Hippodamia convergens Guerin-Meneville, is a specialized predator of cereal aphids on the High Plains, completing its first generation each year in winter wheat, the resulting adults dispersing into summer crops and producing additional generations, contingent on the availability of aphids. In the present study, we tested the collective value of supplementary plant resources (sugars, pollen, and seedling wheat leaves), and small amounts of alternative prey, eggs of Ephestia kuehniella (Zeller) (Lepidoptera: Pyralidae), all provided together, for improving beetle life history and reproductive success even when suitable prey, Schizaphis graminum (Rondani) (Hemiptera: Aphididae), were provided ad libitum. Although a monotypic diet of S. graminum yielded slightly faster larval development and heavier adults than the omnivorous diet, preoviposition periods were extended and 21-d fecundities greatly reduced when this diet was continued through adult life, largely due to fewer oviposition days, although egg fertility was unaffected. The results highlight the critical importance of plant-derived resources even when suitable prey are not limiting. However, monotypic diet beetles that diapaused for 21 d in the presence of supplementary plant resources, plus moth eggs, achieved the same reproductive success as those reared on the omnivorous diet, with or without diapause, demonstrating that access to these resources postemergence was sufficient to compensate for their absence during development. The diapause treatment itself had no impact on the fitness of

beetles reared on the omnivorous diet, likely because neither its duration, nor the caloric restriction imposed, were sufficient to diminish reproductive effort.

2.2 | INTRODUCTION

Lady beetles (Coleoptera: Coccinellidae) are ubiquitous predators of aphids and other pests of cereal crops on the High Plains and have long been recognized as important biological control agents in many agroecosystems around the world (Obrycki and Kring 1998, Schmidt et al. 2003, Brewer and Elliot 2004, Weber and Lundgren 2009, Michaud 2012). They generally exhibit high mobility in the landscape (Bianchi et al. 2009, Jeffries et al. 2013), tolerate agricultural disturbances relatively well (Sloggett and Majerus 2000), and exhibit generalist feeding habits that may improve their effectiveness as biological control agents (Sloggett and Majerus 2000, Hodek and Honek 2009, Schuldiner-Harpaz and Coll 2017). Although lady beetles are typically characterized as predators, and carnivory is thought to be their ancestral condition (Giorgi et al. 2009), they also exploit many food sources of plant origin (Sloggett and Majerus 2000, Lundgren 2009). The consumption of nonprey foods such as pollen, nectar, and tender plant tissues may assist with both hydration and nutrition, enabling survival in times of prey scarcity, a common situation for species that prey on ephemeral insects like aphids (Hodek 1996, Hodek and Evans 2012). Dietary components of plant origin are also utilized when prey is plentiful, likely because they provide supplementary nutrients that can improve developmental and reproductive success when these are limiting (Berkvens et al. 2010, Choate and Lundgren 2013, Radonjic et al 2018). Therefore, the consumption of supplementary foods (Sensu Michaud 2005) is not just compensatory behavior but an important part of the natural diet of predatory

coccinellids (Schuldiner-Harpaz and Coll 2017, Radonjic et al. 2018, Hatt and Osawa 2019, Ugine et al. 2019).

Lundgren (2009) argued that more diverse diets were generally beneficial for larval development and adult weight at emergence, and that access to sugar sources was also beneficial for adult reproduction. Because nonprey foods such as pollen and sugar sources are relatively common in agroecosystems, conservation biological control programs would benefit from a better understanding of how this omnivory affects the performance and fitness of important biological control agents. On the High Plains, coccinellids inhabit mosaic of agricultural habitats, typically developing in one crop and then migrating to reproduce in another (e.g., Bastola et al. 2016). In the process, they switch among prey types and supplementary foods, depending on their availability (Michaud 2018). Because of their roles in biological control, we tend to focus on evaluating their performance on target pest species, and often rear them on diets of monotypic prey, but their actual performance in nature may vary in accordance with their ability to access a more omnivorous, diverse, and nutritionally balanced diet.

Hippodamia convergens is a coccinellid native to the western hemisphere with a broad distribution across Nearctic and Neotropical regions. Populations inhabiting the High Plains region are highly adapted to local conditions (Michaud and Qureshi 2006) and contribute significantly to biological control of aphids, as well as eggs and early juvenile stages of other soft-bodied agricultural pests, in cereal and forage crops (Rice and Wilde 1988, Nechols and Harvey 1998, Colares et al. 2015). Although this species relies on access to abundant aphids for reproduction (Vargas et al. 2013), its life history can benefit from access to supplementary foods. For example, Mercer et al. (2020) demonstrated the life history benefits to overwintering adults of access to winter supplements of pollen, sugar sources, and other supplementary foods. In the

present study, we examined the effects of an omnivorous diet on H. convergens development and reproduction, in contrast to a diet of monotypic prey as might typically be fed to a laboratory colony. In the mixed diet treatments, we provided supplementary food sources, or their analogs, which would mimic those naturally available to first generation H. convergens. These beetles develop feeding primarily on aphids in wheat, and once mature, either move directly to reproduce on aphids in a summer crop or enter a period of reproductive diapause due to aphid scarcity, eventually reproducing at some later date. We selected the greenbug, Schizaphis graminum (Rondani), as aphid prey, as it occurs on both winter (wheat) and summer (sorghum) crops. A relatively short (21 d) diapause treatment was overlaid on the diet treatments, because aphid scarcity is common after maturation of the first spring generation, and nutrients obtained by adults during the diapause period are stored in fat bodies rather than being used immediately for egg production (Michaud and Qureshi 2005).

Specifically, we asked whether monotypic larval diets of S. graminum aphids, versus mixed diets containing both prey and supplementary plant foods, would affect larval development, adult body size, and reproductive performance (i.e., fecundity and fertility). We hypothesized that H. convergens larvae fed an omnivorous diet would show faster development and higher adult body weight than those fed a monotypic diet. In addition, we hypothesized that H. convergens adults reared on a monotypic larval would suffer reduced reproductive success if denied the opportunity for omnivory for their entire lives. A period of reproductive diapause, during which supplementary plant resources were provided in conjunction with small amounts of non-aphid animal protein (eggs of Ephestia kuehniella (Zeller)), was predicted to compensate for the deprivation of these resources during larval development.

2.3 | MATERIALS AND METHODS

2.3.1 Stock Insect Colonies

Adult Hippodamia convergens were collected at the Agricultural Research Center-Hays, Kansas State University, in Hays, Kansas (38°51′32.1″N 99°20′07.7″W) in late May 2019 and placed in a 1 liter glass mason jar (ca. 150 beetles) covered with muslin cloth. The jar was filled with strips of wax paper for harborage, water was provided on a cotton wick, and a few mg of frozen eggs of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae) were added every other day. The colony was held in a climate-controlled chamber set to 22 ± 1°C, 50–60% RH, and a 16:8 (L:D) h photoperiod and monitored daily for 2 weeks to ensure that all beetles parasitized by Dinocampus coccinellae Schrank (Hymenoptera: Braconidae) could be removed.

A colony of greenbugs was established on wheat seedlings grown in a mixture of soil:peat moss:perlite (1:1:1) in metal trays in a greenhouse. Trays of wheat seedlings were infested at the two-leaf stage by brushing aphids from an older tray onto the soil surface. Infested trays were held in a plant growth chamber set to 24 ± 1°C, 50–60%, and a 14:10 (L:D) h daylength under fluorescent lighting.

2.3.2 Experimental Design

The experiment was held under the same physical conditions as the stock colony. Female H. convergens (n = 25) from the stock colony were isolated in plastic Petri dishes (5.5 cm diam) and provided greenbugs ad libitum, refreshed daily, to induce oviposition. After successful eclosion of egg clusters was verified, a single day's oviposition of 17 fertile females was used to generate larvae for the experiment, each female contributing equal numbers of larvae to each treatment. The maternity of all experimental insects was tracked to avoid the subsequent pairing of siblings. Eclosing first instar larvae were isolated in Petri dishes (as above) after they began to

disperse from their cluster. This allows natural consumption of chorions and avoids harming teneral larvae; any larvae observed cannibalizing unhatched eggs were discarded, as this can affect developmental times (Bayoumy and Michaud 2015).

Neonate larvae were divided into two treatment groups for rearing, ensuring that the offspring of each female were equally represented in each. One group was fed only greenbugs ad libitum (n = 108), without any plant material (henceforth, the monotypic diet). The other group received greenbugs (n = 109) on the excised leaves of their host plant (wheat seedlings), frozen eggs of E. kuehniella (ca. 5–10 mg), some pulverized bee pollen (ca. 5–10 mg), and diluted honey (30% honey by volume) on a small cube of sponge (henceforth, the omnivorous diet). These dietary supplements were selected as analogs of plant-derived resources that would be naturally available to first generation H. convergens larvae as they developed in wheat fields, although pollen would be available only during the flowering period. All foods were refreshed every 24 h until the insects pupated and total larval development and pupation times were recorded daily. Beetles were observed consuming all the various foods during daily observations and were never observed to deplete any of them in any replicate. A single larva died prior to pupation in each treatment.

Within a few hours of emergence, all adults were sexed and weighed on an analytical balance (Mettler Toledo, AG285, Columbus, OH). Beetles from each of the two rearing treatments were isolated in plastic Petri dishes (as above) and then each was further subdivided into two adult feeding treatments to yield a total of four treatments, with care taken to assign equal numbers of unrelated males and females to each. Couples (n = 24 per treatment) were held in plexiglass snap-cap vials (5 cm diam × 10 cm ht) that were ventilated by means of a screened hole in the top. Treatment 1 (T1) received the same monotypic diet as larvae (greenbugs only) to

induce immediate reproduction following maturation and mating. Treatment 2 (T2) consisted of monotypic larval diet beetles that were forced into reproductive diapause for 21 d by removing greenbugs from the diet. A period of diapause is the norm for this species on the High Plains, as beetles emerging from maturing wheat do not immediately have access to aphids on summer crops. We selected 21 d as a period sufficient to induce a full reproductive diapause, but not so long that adults would begin breaking diapause and reproducing without access to aphids, as they eventually do (Michaud and Qureshi 2006). During this period, beetles were provided with supplementary resources that that were denied as larvae (leaves of sorghum seedlings, pulverized bee pollen, frozen eggs of E. kuehniella, and diluted honey), plus sunflower stem segments sealed on each end with melted paraffin to delay desiccation. Sorghum replaced wheat to correspond to a summer host plant for greenbugs, and sunflower extrafloral nectar is a resource typically utilized by H. convergens adults encountering prey scarcity under hot, dry summer conditions (Michaud and Qureshi 2005). After the diapause period, during which no females laid eggs, greenbugs were again provided ad libitum to induce oviposition. Treatment 3 (T3) consisted of omnivorous larval diet beetles that received the same adult omnivorous diet as T2, but with the addition of ad libitum greenbugs, from emergence onward. Treatment 4 (T4) was the same as T3, except that beetle pairs were forced into diapause for 21 d, as described for T2, before having greenbugs reintroduced to induce oviposition (Figure 3). It was not feasible to impose a treatment that included reproductive diapause without access to supplementary foods because beetles cannot survive without access to proteinaceous food for more than 7 d, on average (Michaud and Qureshi 2005).

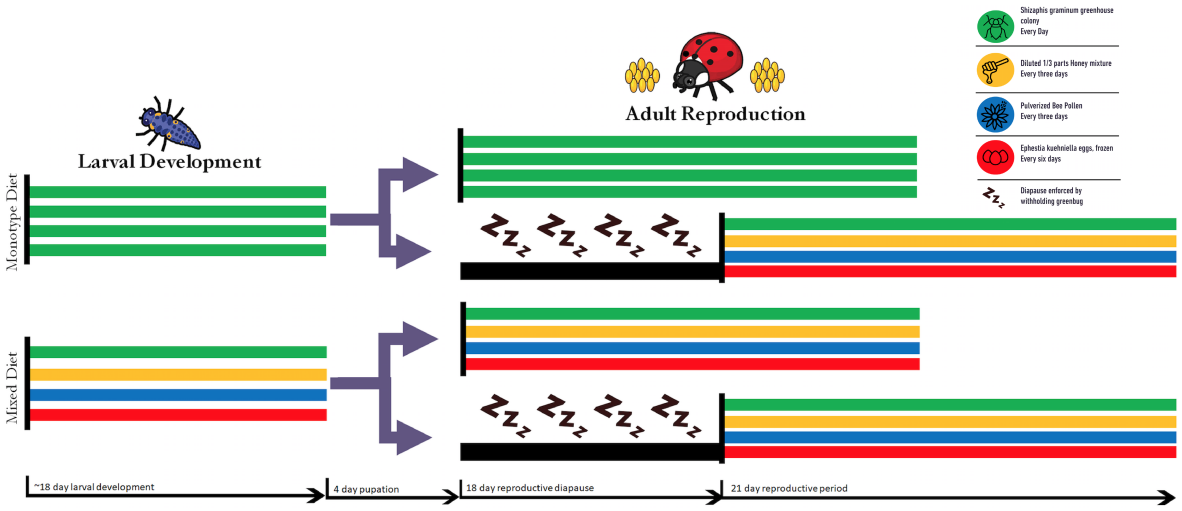

Figure 3 Diet composition and timeline for larval and adult life stages. Monotypic diet comprised of only *Schizaphis graminum* aphids (green), Mixed diet comprised of *S. graminum*, *Ephestia kuehniella* moth eggs (red), diluted honey as a nectar analog (yellow), and mixed pulverized bee pollen (blue). Reproductive diapause (black) enforced by withholding *S. graminum* aphids which is necessary for oocyte maturation.

Mating pairs were held together for the duration of the experiment to ensure maximum female fertility. Eggs were collected daily by switching beetles to clean containers; all food resources were refreshed at this time. Eggs were monitored until eclosion to determine the percentage that hatched as an assay of the fertility of each couple. All egg production, and number of oviposition days, were tallied for 21 d, counted from the first day of oviposition for each female. One female in T1 died on her last day of oviposition, and one male in T1 died on the third-to-last oviposition day for that couple, so these data were considered valid and were not excluded analysis.

2.3.3 Statistical Analysis

Developmental data consisted of only two treatments, so a simple one-way ANOVA was used to compare treatment effects on duration of larval development, pupation time, and adult fresh weight at emergence. Separate two-way ANOVAs were used to determine whether 'diet'

and ‘diapause’ interacted to affect reproductive response variables (no. reproductive days, 21-d fecundity, and egg fertility). Our experimental design yielded a series of four preplanned comparisons with respect to data on adult fecundity and fertility, and the preoviposition period (POP) in the case of non-diapausing treatments. Contrasts were as follows: T1 versus T2 would test whether the diapause treatment had any effect on the reproductive performance of beetles that developed on the monotypic diet of greenbugs; T1 versus T3 would reveal any reproductive benefits of the omnivorous diet during development, in the absence of any period of adult diapause; T2 versus T4 would reveal any reproductive benefits of the omnivorous larval diet when adults experience a period of diapause prior to reproduction. Finally, T3 versus T4 would reveal whether the adult diapause treatment had any effect on reproductive success when beetles were reared on the omnivorous larval diet. All other contrasts were considered unjustified, as more than one independent factor differed between them. All analyses were performed using SPSS statistical software (SPSS 2009).

2.4 | RESULTS

Diet treatment had a significant effect on larval developmental time ($F_{1,211} = 203.9$; $P < 0.001$) and on pupal development time ($F_{1,211} = 19.0$; $P < 0.001$). Although beetles raised on the monotypic greenbug diet developed significantly faster than those on the omnivorous diet, the latter spent less time in the pupal stage (Fig. 4). Because the difference between treatments in pupation time was much smaller than the difference in larval development time, adults still emerged earlier in the monotypic diet treatment. They also emerged with greater fresh weight in the monotypic diet treatment (Fig. 5), whether male ($F_{1,85} = 104.6$; $P < 0.001$), or female ($F_{1,114} = 185.3$; $P < 0.001$).

The two-way ANOVA revealed significant main effects of diet ($F_{1,83} = 26.6$; $P < 0.001$), and diapause ($F_{1,83} = 12.7$; $P = 0.001$) on fecundity, and a significant interaction between these factors ($F_{3,83} = 8.6$; $P = 0.004$). A monotypic diet of greenbugs that continued through larval development into adult life without diapause significantly reduced 21-d fecundity compared with either the same larval diet with diapause, or an omnivorous diet without diapause (Fig. 6). However, there was no effect of either diet ($F_{1,83} = 0.7$; $P = 0.414$) or diapause ($F_{1,83} = 0.1$; $P = 0.751$) on egg fertility, which averaged 71.6 ± 3.2% experiment-wide, and no significant interaction term ($F_{3,83} = 0.1$; $P = 0.458$). Much of the difference in fecundity could be explained by differences in the number of reproductive days over the 3-wk observation period ($y = 0.0252x + 4.172$, $r2 = 0.753$), which responded to both diet ($F_{1,83} = 18.2$; $P < 0.001$) and diapause (F1,83 = 11.2; $P < 0.001$), with a significant interaction between them ($F_{3,83} = 6.0$; $P = 0.016$). A lifetime monotypic diet of greenbugs, without diapause, resulted in significantly fewer days of oviposition in 3 weeks compared with the monotypic diet with diapause ($F_{1,38} = 15.7$, $P < 0.001$), or the omnivorous diet without diapause ($F_{1,42} = 22.8$, $P < 0.001$, Fig. 7). However, beetles on the monotypic larval diet with diapause did not differ in number of oviposition days from the omnivorous diet with diapause ($F_{1,41} = 1.6$, $P = 0.210$), which in turn did not differ from the omnivorous diet without diapause ($F_{1,41} = 1.6$, $P = 0.210$).

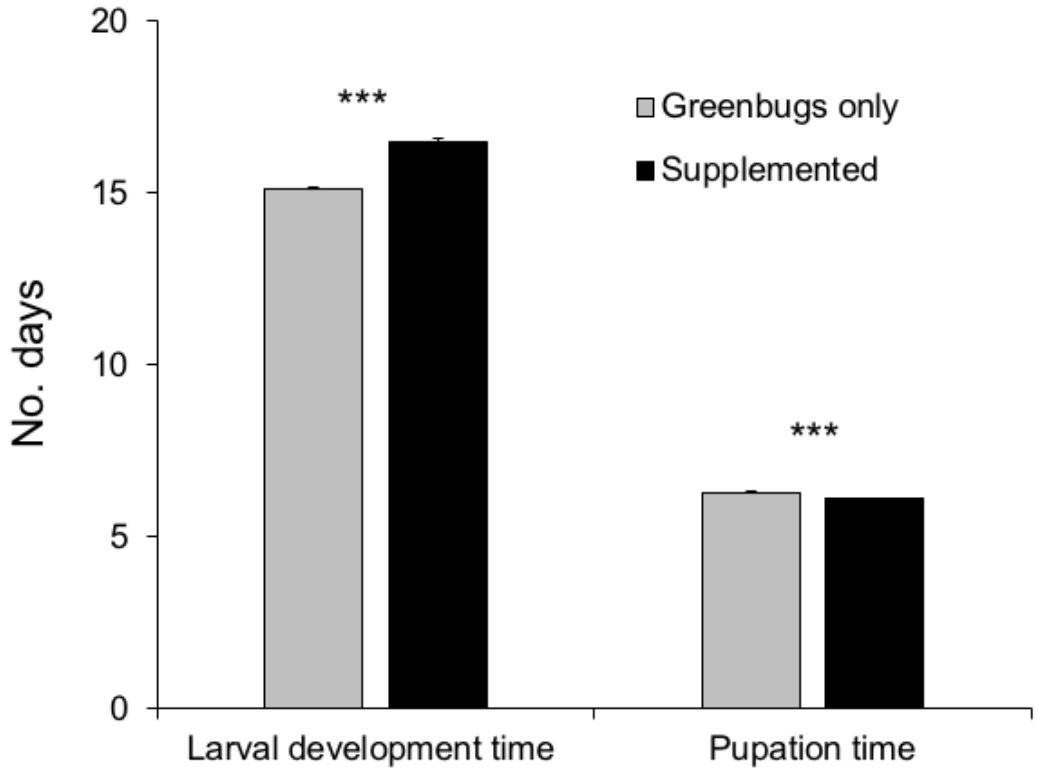

Figure 4 Mean (+SE) number of days required for Hippodamia convergens to complete larval development and pupation when reared on a monotypic diet of greenbugs (Greenbugs only), Schizaphis graminum, versus an omnivorous diet containing greenbugs, wheat leaves, pulverized bee pollen, diluted honey, and frozen eggs of Ephestia kuehniella (Supplemented). Asterisks indicate a significant difference between diet treatments (*, ANOVA, P < 0.001).**

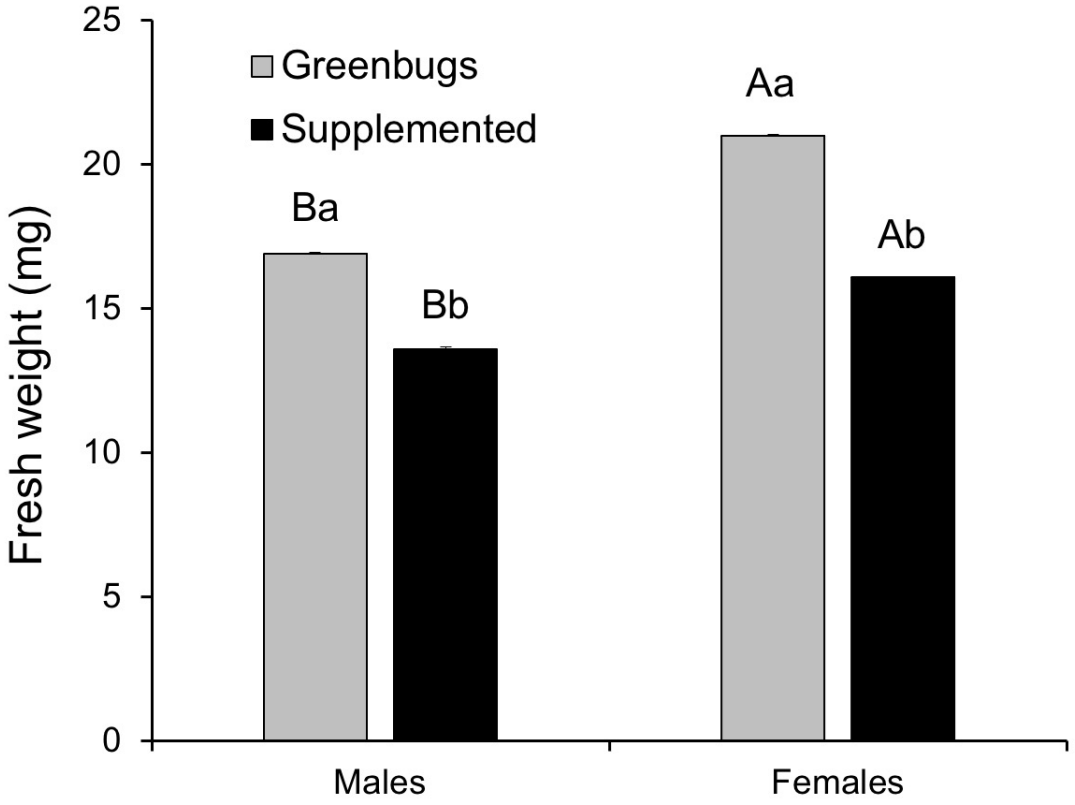

Figure 5 Mean (+SE) adult fresh weights of male and female Hippodamia convergens at emergence when reared on a monotypic diet of greenbugs (Greenbugs), Schizaphis graminum, versus an omnivorous diet containing greenbugs, wheat leaves, pulverized bee pollen, diluted honey, and frozen eggs of Ephestia kuehniella (Supplemented). Values bearing different upper case letters were significantly different between genders within diets; values bearing different lower case letters were significantly different between diets, within genders (ANOVA, $\alpha = 0.05$).

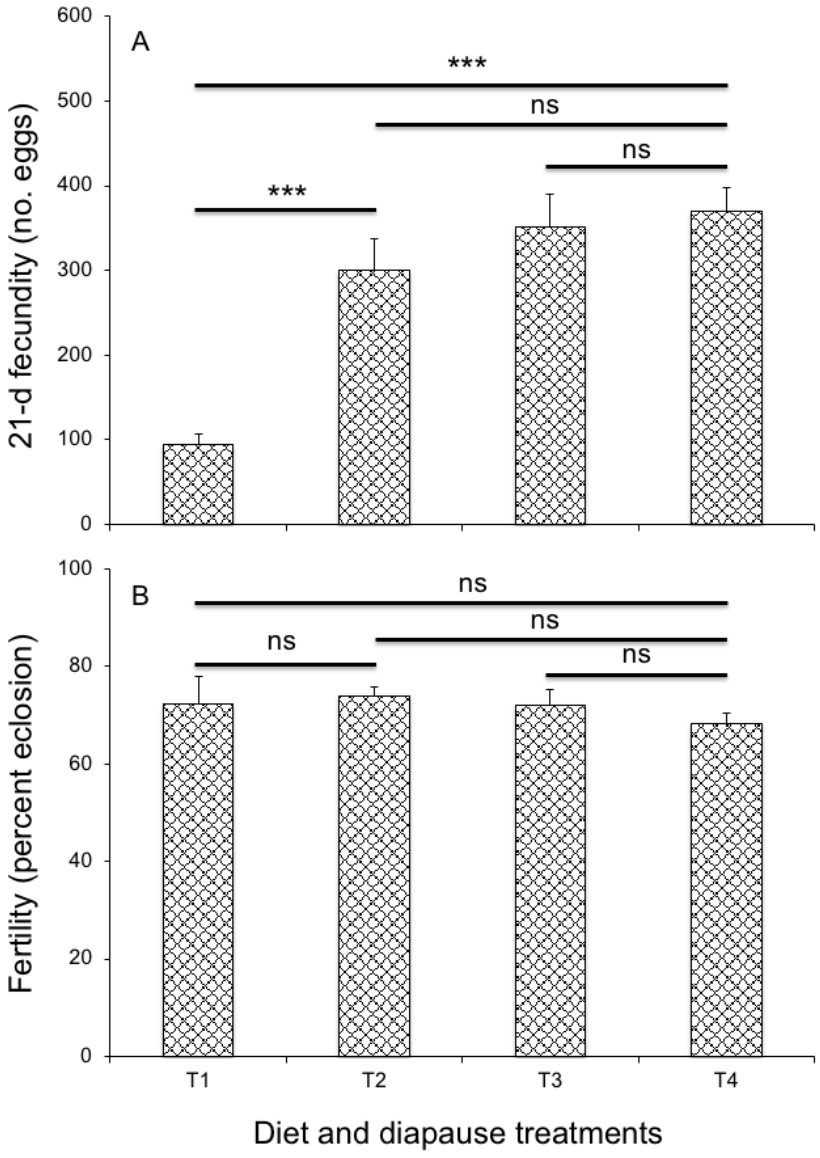

Figure 6 Mean (+SE) 21-d fecundities of Hippodamia convergens pairs subjected to four different diet treatments. T1: development and reproduction on a monotypic diet of Schizaphis graminum, without diapause; T2: development and reproduction on a monotypic diet of Schizaphis graminum, with a 21-d diapause period prior to oviposition; T3: development and reproduction on an omnivorous diet, without diapause; T4: development and reproduction on an omnivorous diet, with a 21-d diapause period prior to oviposition. Preplanned, pairwise comparisons among treatments (*, ANOVA, P < 0.001).**

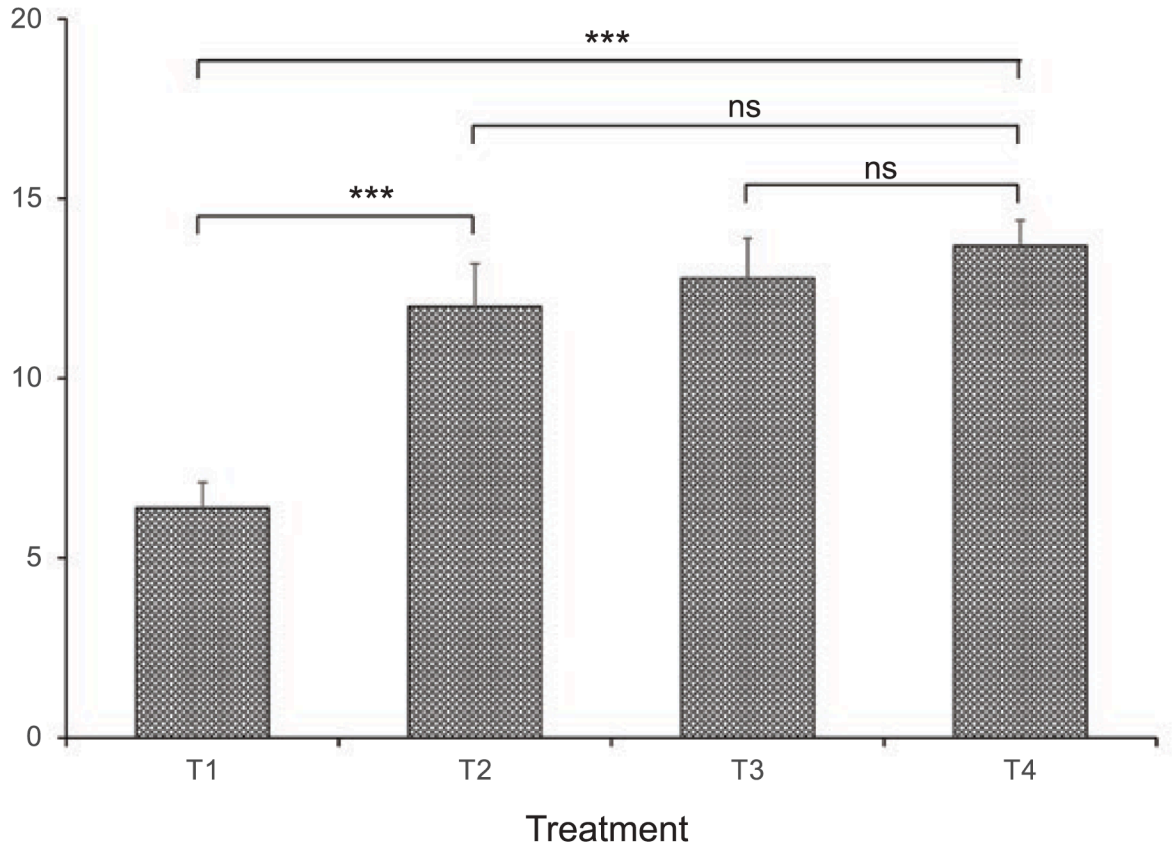

Figure 7 Mean (+SE) number of oviposition days by Hippodamia convergens females when subjected to four different diet treatments and observed for 21 d after first oviposition. T1: development and reproduction on a monotypic diet of Schizaphis graminum, without diapause; T2: development and reproduction on a monotypic diet of S. graminum, with a 21-d diapause period prior to oviposition; T3: development and reproduction on an omnivorous diet, without diapause; T4: development and reproduction on an omnivorous diet, with a 21-d diapause period prior to oviposition. Preplanned, pairwise comparisons among treatments (*, ANOVA, P < 0.001).**

2.5 | DISCUSSION

We hypothesized that H. convergens larvae fed an omnivorous diet would show faster development compared with beetles on the monotypic diet. Instead, we found slower larval development on the omnivorous diet compared with the monotypic diet, which likely reflects the fact that consumption of supplementary plant resources displaced some intake of animal protein and lipids on which growth rate depends. A higher dietary protein intake relative to carbohydrate

generally correlates with shorter developmental times in coccinellids (de Clercq et al. 2005, Jalali et al. 2009, Hodek and Evans 2012), even in herbivorous species (Wang et al. 2018). In the present study, the slightly faster pupation time in the omnivorous diet treatment may indicate a beneficial effect of plant-derived nutrients on pupal development, even though this diet was associated with a smaller body size at emergence. These results indicate that consumption of plant-derived resources during the larval stage incurs small costs in terms of developmental time and body size at emergence compared to an aphid-only monotypic diet.

Our results supported the hypothesis that an omnivorous diet treatment would increase fecundity relative to development and reproduction on a monotypic diet, although our experimental design did not resolve the individual contributions of each of the nonprey dietary components. Pairs of H. convergens that remained on the monotypic diet as adults produced far fewer eggs than those in other treatments over 21 d of reproduction, largely as a result of fewer female oviposition days, even though egg fertility was similar across all treatments. Hippodamia convergens is an aphid specialist, and aphid availability appears to be the primary factor limiting fecundity in such species (Vargas et al. 2013), as ovariole maturation by females is minimal on alternative prey such as eggs of E. kuehniella, unless they have consumed aphids during the larval stage (J.P.M., personal observations). However, our results indicate that nutritional factors of plant origin are critical to maximize fecundity in this species, although apparently not necessary to ensure egg fertility. Phytosterols are a prime candidate in this regard; insect herbivores must obtain appropriate phytosterols in their diet to convert into cholesterol, as they are unable to directly synthesize this important biological molecule (Levinson 1962). Furthermore, phytophagous insects vary greatly in their ability to utilize different phytosterols

(Svoboda and Feldlaufer 1991), and predators could presumably supplement their own phytosterol requirements via omnivory.

When beetles diapaused with supplemental foods as adults, the monotypic larval diet had no detrimental effect on adult reproduction. This suggests that consumption of supplemental foods by adults during the maturation stage, or in diapause, can compensate for limited omnivory during larval stages, something that might arise due to their limited mobility. This finding also has potential utility for mass-rearing, as developmental rate and body size could be maximized by rearing larvae exclusively on aphid prey, and avoiding the detrimental effects of this diet on reproductive performance by providing the emerging adults with access to supplemental plant resources.

These results also indicate that supplemental plant resources are consumed by H. convergens larvae even when prey are available ad libitum, i.e., not only in the absence of prey. Although considerable previous work has demonstrated the importance of pollinivory to coccinellid development and survival (Hodek 1996, Jalali et al. 2009, Lundgren 2009, He and Sigsgaard 2019), much less information exists on its contribution to reproductive performance, especially when prey are not limiting. For example, pollinivory can accelerate the onset of oviposition in Adalia bipunctata L. when aphids become available. Coleomegilla maculata DeGeer is perhaps unique among predaceous coccinellids in being capable of very limited reproduction on an exclusive diet of pollen (Lundgren and Wiedenmann 2004). However, supplementation of the adult diet of this highly polyphagous species with either corn or sorghum pollen does not improve either fecundity or fertility over a monotypic diet of Ephestia eggs, despite many plant-specific effects of pollen on development (Michaud and Grant 2005). More

recently, Pilorget et al. (2010) demonstrated that interspecific variation in suitability of plant pollens for C. maculata correlates with their phytosterol content.

Although both adults and larvae of many species have been observed consuming the tender foliage of various plants (Hodek 1973, Moser et al. 2008, 2009; Hodek and Evans 2012), there exists less information on the potential contributions of folivory to coccinellid fitness. An illuminating study of Coccinella septempunctata L. by Ugine et al. (2019) found that this species experiences spermatogenic failure when denied access to plant material, which can be corrected by herbivory and dietary supplementation with phytosterols and cholesterol. The authors concluded that folivory in this species is driven by a sterol-specific appetite associated with a physiological deficit of sterols. It seems likely that other coccinellid species, including H. convergens, have their own specific phytosterol requirements, and the various possible fitness limitations resulting from phytosterol deficiencies are worthy of further investigation.

The availability of diluted honey in the omnivorous diet treatment provided a source of supplementary carbohydrates analogous to flower nectar, which may be valuable to adults as a source of energy for flight, and thus important for effective foraging in nature. Such benefits may pass unnoticed in a laboratory setting where there is no need for dispersal to find patches of prey, nor any opportunity for flight. Floral nectar, extrafloral nectar (Schuster et al. 1976), and sugar sprays (Carlson and Chiang 1973, Mensah and Madden 1994, Evans and Richards 1997, van der Werf et al. 2000) have all been shown to attract and retain predatory coccinellids in field studies. A meta-analysis of published work by Lundgren (2009) concluded that sugar sources often enhance the reproductive performance of various predatory coccinellids on prey-only diets and were important dietary constituents for maximizing fitness. He and Sigsgaard (2019) found that sugar extended survival in A. bipunctata larvae in the absence of prey and increased the lipid

content in adults even when prey were available. There is also evidence to suggest potential impacts of carbohydrates on reproductive success. For example, provisioning with sugar, as opposed to water alone, shortens the preoviposition period of the acarophagous Stethorus japonicus Kamiya and reduces the amount of prey (eggs of Tetranychus urticae Koch) required to induce the onset of oviposition (Kishimoto and Adachi 2010). Provisioning of honey together with prey has been shown to increase fecundity in comparison to diets that exclude carbohydrates in Cryptolaemus montrouzieri Mulsant (Marques 2015) and C. maculata (D'Avila et al. 2017).

We found no effect of the diapause treatment on the reproductive performance of H. convergens that were reared on the omnivorous diet. Reproductive diapause is an adaptation that adult H. convergens use to survive periods of aphid scarcity, when insufficient prey are available to support either adult reproduction or larval development. Any meager amounts of animal protein (e.g., moth eggs) obtained during this period are stored in fat bodies for later use (Michaud and Qureshi 2005, Hodek 2012, Hodek and Evans 2012). Pollen, being high in plant protein and phytosterols, also supports survival during diapause (Ceryngier et al. 2004, Lundgren 2009, Hodek and Evans 2012), so access to pollen becomes more important during periods of prey scarcity. Hatt and Osawa (2019) showed that increased access to flowers of Perilla frutescens (Lamiaceae) improved the longevity of Harmonia axyridis Pallas adults and enabled an earlier peak in fecundity, even though overall fecundity was not different from the prey-only diet. However, extended diapause eventually exacts a cost on fecundity, as resources that could have been used for reproduction are diverted to prolong adult lifespans (Michaud and Qureshi 2005). In the present study, it seems likely that neither the caloric restriction imposed, nor the period of diapause, were sufficient to exact a measurable cost on female fecundity.

In conclusion, our results highlight the importance of access to supplementary resources, of both plant and animal origin, for maximization of fitness in H. convergens, even though this species is considered a specialized aphid predator. Whether these resources are acquired during larval development or during a prereproductive adult diapause, they have similar benefits for reproductive success, even though their consumption during the larval stage causes slightly delayed development and reduced adult body size. It follows that cultural approaches to preserving or improving biological control of aphids by H. convergens should seek ways to provision first generation adults with access to a diversity of floral resources as they leave maturing wheat fields and either begin reproduction or enter a period of reproductive diapause until aphids become available on summer field crops.

2.6 | REFERENCES

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Chapter 3 - Floral Resources Enhance Fecundity, but not Flight Activity in a Specialized Aphid Predator, *Hippodamia convergens* (Coleoptera: Coccinellidae).

3.1 | ABSTRACT

Adult aphid predators disperse across the landscape seasonally in search of prey aggregations that are patchily distributed and temporally variable. However, flight is energetically costly and consumes resources that could be invested in reproduction. *Hippodamia convergens* is an important aphid predator in North American cereal crops and other agricultural systems. Consumption of floral resources can enhance adult survival during periods of low prey availability and may improve reproductive success. We tested how an omnivorous adult diet containing floral resources (diluted honey and pulverized bee pollen) interacts with body size to influence reproduction and flight behavior compared to a prey-only diet. Two sizes of beetles were produced by controlling larval access to food – 3h daily access produced small beetles; *ad libitum* access produced large beetles with faster development. Reproductive performance was tracked for 18 days, and female flight activity was assayed via 3h bouts of tethered flight. Diet composition and body size interacted to influence preoviposition period, with large females in prey-only treatments delaying oviposition the longest. The omnivorous adult diet improved 18 d fecundity relative to a prey-only diet, but egg fertility was unaffected. Adult size affected oviposition pattern, with small beetles laying smaller, but more numerous, clutches. Females flew up to 7 km in 6h, but neither body size nor adult diet influenced flight distance, suggesting that all diet treatments generated energy reserves sufficient to power flights of short duration. However, pre-reproductive females flew > 60% further than they did post-reproduction, likely

due to the energetic costs of oviposition. Thus, access to pollen and nectar increased reproductive success and altered oviposition patterns in *H. convergens*, indicating the importance of floral resources in the agricultural landscape to conservation of this predator and its biological control services.

3.1.1 Keywords: biological control, life history, energetic trade-offs, insect predators, flight capacity, agricultural ecology, omnivory

3.2 | INTRODUCTION

The convergent lady beetle, *Hippodamia convergens* Guerin-Meneville, is a generalist insect predator that provides biological control services to agriculture throughout North America. It is primarily a predator of aphids, which adults require for reproduction (Hagen, 1962; Michaud and Qureshi, 2006), but it also preys upon the immature stages of many other agricultural pests as both larva and adult (Hodek, 1996; Michaud, 2018). It has long been associated with agricultural crops in North America, where it has proven adaptable to frequent disturbance, low plant diversity, and other characteristics of industrial agriculture (Hagen, 1962; Sloggett and Majerus, 2000). Predacious coccinellids, in general, appear well adapted to agroecosystems and often persist there at higher densities than in adjacent natural areas (Hagen, 1962; Lamb et al., 2019). In agriculturally intensive regions of North America, large fields host successions of annual monocultures seasonally, their planting dates dictated by each crop's thermal and hydrological requirements. At landscape scale, this results in an agricultural mosaic of resource patches for arthropod herbivores and their predators that is both spatially variable and temporally dynamic across growing seasons (Bianchi et al., 2009). On the High Plains, adult *H. convergens* track aphid populations across the landscape (e.g., Prasifka et al., 2004), moving predictably between crops such as wheat and alfalfa, where overwintered beetles produce a spring generation, to summer crops such as corn and sorghum, where additional generations occur, contingent on the availability of aphids (Rice and Wilde, 1988; Nechols and Harvey, 1998; Michaud and Qureshi, 2006). They are also able to survive extended periods of prey deprivation by consuming various plant-derived resources, including tender green foliage, pollen, and nectar of both floral and extra-floral origin (Hodek, 1996; Michaud and Qureshi, 2005; 2006; Mercer et al., 2020). However, utilization of floral resources in an agricultural landscape also carries a risk

of exposure to systemic insecticides now widely employed as seed treatments (Gontijo et al., 2014; Moscardini et al., 2015; Bredeson and Lundgren, 2018) in addition to foliar applications (He et al., 2012).

Supplemental foods of plant origin not only support the survival of predaceous coccinellids in the absence of animal prey (Hodek, 1996; Hatt and Osawa, 2019) they are often necessary for the beetles to achieve maximal fitness (Lundgren, 2009; Michaud, 2018; Stowe et al., 2021). Consequently, these resources are also consumed during periods of prey abundance (Berkvens et al., 2010; Hodek and Evans, 2012; Choate and Lundgren, 2013; Ugine et al., 2019), and may be selectively consumed when particular nutrients are lacking in the diet (Schuldiner-Harpaz and Coll, 2017). The inclusion of plant resources in the diet of aphidophagous coccinellids can facilitate earlier onset of oviposition (Hatt and Osawa, 2019) and enhance egg fertility (De Clercq et al., 2005; D'Avila et al., 2017). Dietary self-selection of foods rich in specific macronutrients is well documented in many insects (Jensen et al., 2012), both under normal conditions (Jones and Raubenhiemer, 2001; Mayntz et al., 2005), and in response to physiological deficits (Raubenhiemer and Jones, 2006; Raubenhiemer et al., 2007). Ratios of macronutrients in the diet can influence insect reproduction and flight behavior by affecting the availability of metabolic precursors and titers of metabolites and hormones, as exemplified by anautogenic mosquitos (Attardo et al., 2005; Hansen et al., 2005). In the case of facultatively omnivorous predators such as *H. convergens*, the consumption of plant resources could influence energetic trade-offs between dispersal and reproduction. An improved understanding of any such tradeoffs, and the role of plant-derived nutrients in mediating them, could provide key insights for conserving the biological control services provided by these predators in agricultural landscapes.

Flight is a behavior critical to the survival and fitness of *H. convergens* because adult beetles must track populations of their ephemeral aphid prey across large spatial scales in the agricultural landscape in order to achieve reproductive success (Hagen, 1962; Wissinger, 1997; Michaud, 2012). Dispersal by flight can be critical to biological control efficacy as insect predators move among crops, and flight behavior will have a significant impact on energy budgets (Bonte et al., 2012). Dietary effects on flight capacity could potentially affect the range of beetle movement, and thus the ability of beetle populations to track and control pest populations. As yet, relatively few studies have examined *H. convergens* flight behavior (but see Rankin and Rankin, 1980; Davis and Kirkland, 1982; Abdelwahab et al., 2017), and none have addressed the potential of plant-derived resources to affect flight capacity. The present study was designed to examine how access to floral resources might affect flight capacity and distance flown by *H. convergens* females, and how the energy expended in flight might exact a cost in terms of reproductive performance. Because body size is a key morphological trait that affects both wing loading (Byrne et al., 1988), and potential fecundity (Vargas et al., 2013), we created two size classes of adult beetles by controlling larval access to food. We hypothesized that both larger adult size and access to floral resources would benefit reproductive success and enable beetles to fly longer distances when compared to smaller beetles, and those with access only to prey. We also hypothesized a negative correlation between flight distance and reproductive success, a trade-off that should be more pronounced in smaller beetles with fewer energy reserves. Finally, we used structural equation modeling to explore direct and indirect relationships between adult body size, diet composition, and reproductive and flight metrics to determine if early energy expenditures influence later performance.

3.3 | METHODS

3.3.1 Insect colonies

Adult *Hippodamia convergens* beetles (ca. 120 individuals) were collected from wheat fields at the Agricultural Research Center-Hays, in Hays, Kansas (38°51'32.1"N 99°20'07.7"W). in early June 2020. Beetles were placed in a 1L glass mason jar with a muslin cover and held in a climate-controlled growth chamber set to 24 ± 1 ºC, 50 – 60% RH, and a 16:8 (L:D) photoperiod. Wax paper strips were placed in the jar as harborage, water was provided on a cotton wick, and 10 – 20 mg of frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were added to the jar every 48 hours. Because *H. convergens* are often parasitized by *Dinocampus coccinellae* Shrank (Hymenoptera: Braconidae), the colony was examined daily to remove all emerging parasitoid larvae before they could pupate or emerge as adult wasps. Under these conditions, beetles remain in reproductive diapause and can be held for extended periods (Michaud and Qureshi, 2006).

A colony of greenbug, *Schizaphis graminum* Rondani, was established from material collected from the same wheat field as the beetles. Aphid colonies were reared in metal trays of wheat seedlings in a mixture of soil, vermiculite, and peat moss (1/1/1). Trays were germinated in a greenhouse and then were moved to growth chambers set to 22 ± 1 ºC, 50 – 60% RH, and a 16:8 (L:D) photoperiod under florescent lighting. Plants were infested at the 2-leaf stage by spreading infested wheat cuttings over fresh trays of seedlings.

3.3.2 Experimental Beetles

For the experiment, parental females (n = 29) were isolated in plastic Petri dishes (5.5 cm diam) and held in a climate-controlled growth chamber set to 24 ± 1 C, 50 – 60% RH, and a 16:8

(L:D) photoperiod. Females were provisioned with greenbugs *ad libitum* to induce oviposition and the fertility of each female was confirmed by verifying the eclosion of eggs in her initial clutches. Once a sufficient number of fertile females were ovipositing (n = 29), a single day's oviposition was collected from each for incubation. Neonate larvae were permitted to consume their chorions and disperse from their natal egg mass before they were collected and isolated in Petri dishes (as above). Neonates observed cannibalizing conspecific eggs or other larvae were excluded from the experiment, as cannibalism can alter developmental times (Michaud and Grant 2004, Bayoumy and Michaud 2015). Each larva was labelled according to maternal lineage (n = 29) to ensure that similar numbers of offspring from each lineage were assigned to each treatment (ca. n = 24 couples, per treatment), and that sibling adults were never paired together within treatments.

Larvae were reared on frozen eggs of *E. kuehniella* and provided water on a small cube of sponge, both refreshed daily. Larvae assigned to the 'small adult' treatment were permitted access to food for only 3 h daily (although water remained continuously available), whereas those assigned to the 'large adult' treatment had *ad libitum* access to food. Upon pupation, each petri dish was cleaned of any remaining food to prevent consumption by newly emerged adults and ensure an accurate fresh weight at emergence could be obtained. Adult beetles were all sexed and weighed (within 24 hours of emergence) on an analytical balance (Mettler Toledo, AG285, Columbus, OH). Non-sibling beetles from the same treatment were then paired in ventilated snap cap vials (5 cm diam × 9 cm ht).

Both small adult pairs (n = 48) and large adult pairs (n = 48) were further divided into two groups each, one receiving an omnivorous diet, the other receiving a prey-only diet, both provided *ad libitum*. The omnivorous diet consisted of greenbugs provided on excised leaves of

their host plant, frozen *E. kuehniella* eggs, pulverized bee pollen, dilute honey on a sponge cube (1:2 honey:water), and water on another sponge cube, all provided *ad libitum*. In contrast, the prey-only diet lacked the floral resources and consisted of only greenbugs on wheat foliage, frozen *E. kuehniella* eggs and water on a sponge cube, all provided *ad libitum*. Both greenbugs and water were refreshed daily, whereas *E. kuehniella* eggs, bee pollen, and diluted honey were refreshed every third day. Initially, all beetle pairs were maintained in reproductive diapause for 18 d by withholding the greenbug component of the diet, as female *H. convergens* reared on *E. kuehniella* eggs do not become gravid until provisioned with aphids *ad libitum* for 3 – 4 days (Michaud and Qureshi, 2006). This was done so that we could flight-test all females on a similar time frame both before and after a period of reproduction.

3.3.3 Experimental design

The experiment was organized in a 2×2 factorial design with adult size (large vs. small) and adult diet (omnivorous vs. prey-only) as independent factors, yielding four treatments: (1) small, omnivorous couples (SO, n = 25), (2) small, prey-only couples (SP, n = 24), (3) large, omnivorous couples (LO, n = 25), and (4) large, prey-only couples (LP, n = 25).

3.3.4 Flight Assays

Flight mills (n = 16) were constructed, modified from the design of Attisano et al. (2015), which allowed measurement of total flight distance when connected to recording software (Figure 8). A small dot of magnetic primer (Rust-oleum magnetic primer) was applied to the distal portion of the right elytra of each female beetle using a wooden toothpick. After the paint dried (ca. 24 h), each female was attached to a small neodymium magnet on the end of a flight

mill arm, where she flew at will in a 10cm diameter circle in a climate controlled growth chamber set to 24 ± 1 ºC, 50 – 60% RH. The flight mill arm rotated freely around a frictionless magnetic bearing and distance flown was measured by an infrared light sensor that tracked each revolution of the arm. Data was recorded continuously from the sensors using an Arduino MEGA 2560 REV3 single board microcontroller and terminal program (CoolTerm). For the initial flight test, each female was placed on a mill for a total of 3 hours, then returned to her dish. Following 18 days of oviposition, tallied consecutively from production of her first clutch, each female was flown for another 3h period.

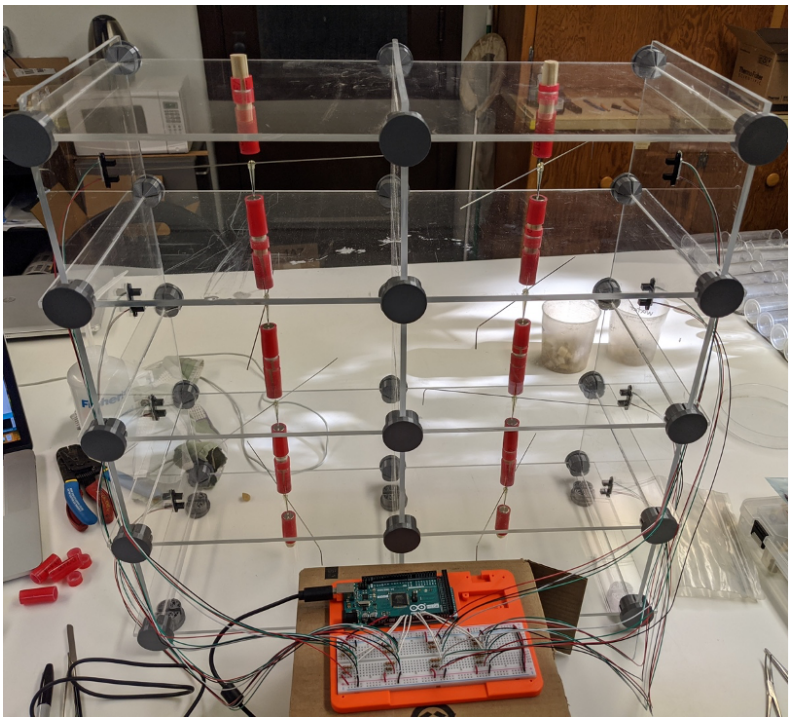

Figure 8 Tethered insect flight mill adapted from Attisano et al. 2015, 2 mill columns x 4 mill rows. Microcontroller and breadboard fitted into a 3D printed tray (orange) with stabilization pegs (charcoal) added at each juncture between vertical and horizontal plexiglass pieces. Sized to fit into a standard growth chamber

3.3.5 Reproduction

Following the addition of greenbugs to the diets, females in both diet treatments were monitored daily for oviposition. Egg clusters, usually laid on the opaque lids of the vials, were collected and held until eclosion under the same physical conditions as the stock colony. The number of eggs in each clutch (daily oviposition) was counted before and after eclosion to obtain total fecundity and egg fertility for each female. This continued for a period of 18 d, a period that is usually sufficient to detect any dietary effects on reproductive success (Michaud, 2005). Females were held together with males from pair formation until the end of the experiment to maximize female fertility. Females that did not oviposit within 30 d after access to greenbugs were concluded to be non-reproductive and completed their second flight test. These non-reproductive females (n = 2) were excluded from reproductive analysis, but included in flight observations, as they expressed the lowest possible reproductive effort.

3.3.6 Statistical analysis

All data passed tests for equality of variance (Levene's test) and normality (Shapiro-Wilk test) and were analyzed with two-way ANOVA, followed by a Bonferroni test to separate means. Preoviposition period was tallied for each female as the no. days from first provision of greenbugs to the first day of oviposition, 18-d fecundity as the total number of eggs laid in 18 days, counted from the first oviposition day, and egg fertility as the percentage of all eggs hatching. Oviposition days were tallied as the total number of days, out of 18, on which at least one egg was laid. Flight distance was tallied as the total number of revolutions completed on the flight mill, multiplied by the circumference of the mill arm. We also used linear regression to test for relationships between pairs of continuous response variables.

Structural equation modeling (SEM) was employed to characterize direct and indirect relationships between independent variables (body size, adult diet) and components of female fitness (preoviposition period, no. of oviposition days, fecundity, egg fertility) and flight performance (pre- and post- oviposition flight distances). Categorical experimental predictors were coded for adult size as 0 (small) and 1 (large), and diet treatment as 0 (prey-only) and 1 (omnivorous). We predicted direct relationships between omnivory / adult size and both reproductive metrics and post-reproductive flight distance (Figure 9, paths A, C, D, H, K, O, P, U). We also wanted to characterize relationships between pre-reproductive flight distance, reproductive performance, and post-reproductive flight distance (paths B, I, L, N, Q). Initial flight distance was included as a predictor in the SEM model selection process because the distribution of initial flight distances indicated intrinsic variation in flight capacity among females. Finally, we were interested in relationships between fitness and flight (paths E, F, G, J, S, V, W, X), specifically, whether tradeoffs existed between energy expended in flight effort or reproduction would diminish subsequent flight and/or reproduction.

Maximum likelihood methods were used for model selection with goodness of fit assessed by Akaike's Information Criterion (AIC) in a step-wise process. Model selection began using the complete hypothesized model with influence between all biologically feasible connections (Figure 9), then removing non-significant factor interactions at each step, beginning with the highest p value. We also used tests of directed separation to identify factors whose addition (or reintroduction) could improve model fit. The AIC value of each successive model version was consulted at each step of the model selection process to assess goodness of fit and models with AIC changes > 2 were discarded. Models with AIC value changes < 2 were

differentiated by parsimony (Grace, 2006; Burnham and Anderson, 2002). R version 4.0.3 was used to conduct all analyses in the piecewise SEM package (Lefcheck, 2016).

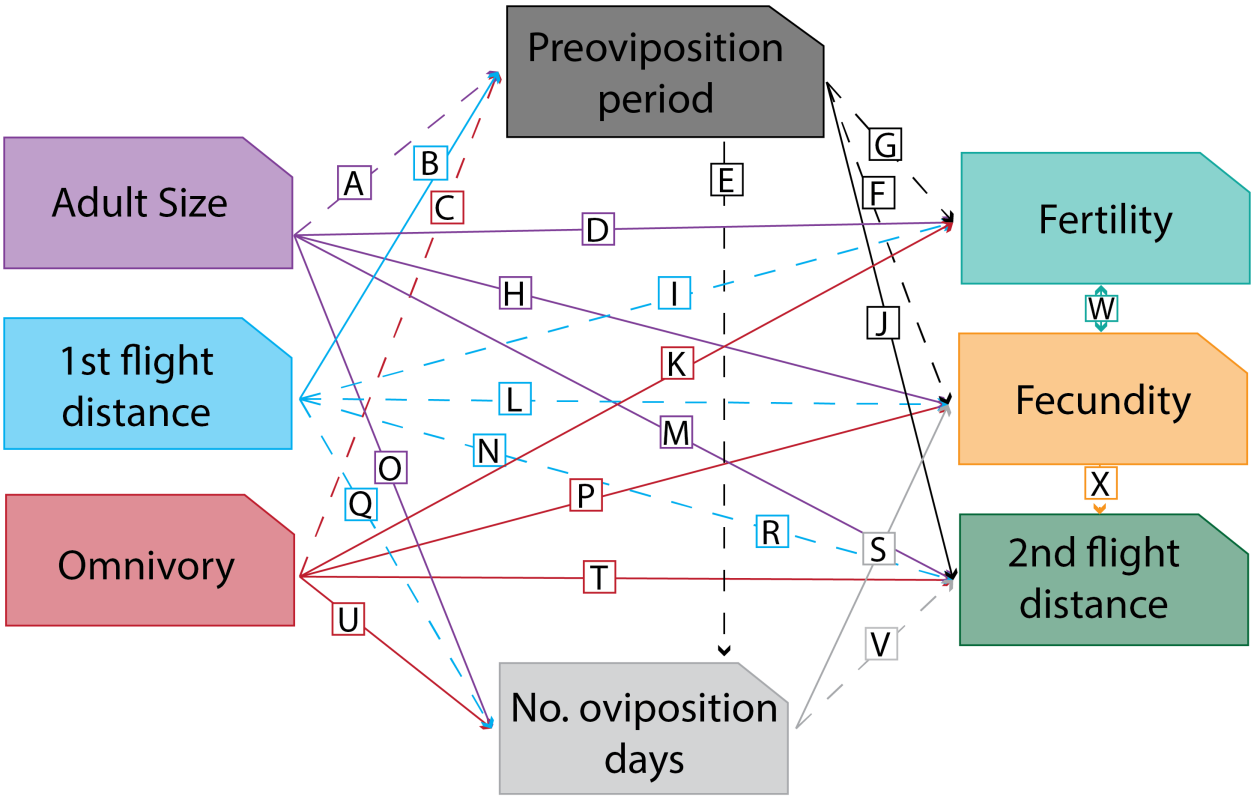

Figure 9 Hypothesis structural equation model: Both larger adult size and omnivory are expected to shorten preoviposition period and increase no. oviposition days, 18-d fecundity, egg fertility and second flight distance. First flight distance is expected to lengthen the preoviposition period and reduce 18-d fecundity, egg fertility, no. oviposition days, and second flight distance. Longer preoviposition period is expected to decrease no. oviposition days, fecundity, egg fertility and increase 2nd flight distance. Increased no. oviposition days is expected to increase fecundity and decrease 2nd flight distance.

3.4 | RESULTS

Larvae restricted to 3 h daily food access were smaller at adult emergence than those reared with *ad libitum* access to food; they weighed less (16.4 mg ± 0.3 vs 21.7 mg ± 0.3, $F =$

119.7; df = 1,97; $P < 0.001$) and developed more slowly (22.5 d ± 0.2 vs 19.0 d ± 0.1, $F = 218.7$; df = 1,97; $P < 0.001$).

Both adult size ($F = 11.01$, df = 1,95; $P = 0.001$) and adult diet ($F = 4.95$, df = 1,95; $P = 0.028$) affected how long females took to begin oviposition after they began receiving greenbugs, but these factors did not interact significantly ($F = 2.47$, df = 1,95; $P = 0.119$). Females in the LP treatment took longer to begin oviposition than females in other treatments, the latter being not significantly different from each other (Figure 10). Similarly, adult size ($F = 5.64$, df = 1,95; $P = 0.020$) and adult diet ($F = 5.43$, df = 1,95; $P = 0.022$) affected the number of oviposition days in the 18 d observation period, again without any significant interaction between factors ($F = 0.001$, df = 1,95; $P = 0.981$). Females in the SO treatment laid clutches on more days than did females in the LP treatment, with other treatments not significantly different (Figure 11). The 18-d fecundity of females was not affected by adult size ($F = 3.30$, df = 1,95; $P = 0.073$) but strongly affected by adult diet ($F = 13.06$, df = 1,95; $P < 0.001$), without any interaction between these factors ($F = 0.91$, df = 1,95; $P = 0.343$). Fecundity was higher in the SO treatment than in either the SP or LP treatments, with no other significant differences among treatments (Figure 12). Egg fertility averaged 62.8 ± 2.9% (mean ± SE) and was unaffected by body size ($F = 2.19$, df = 1,95; $P = 0.142$) or adult diet ($F = 0.16$, df = 1,95; $P = 0.687$).

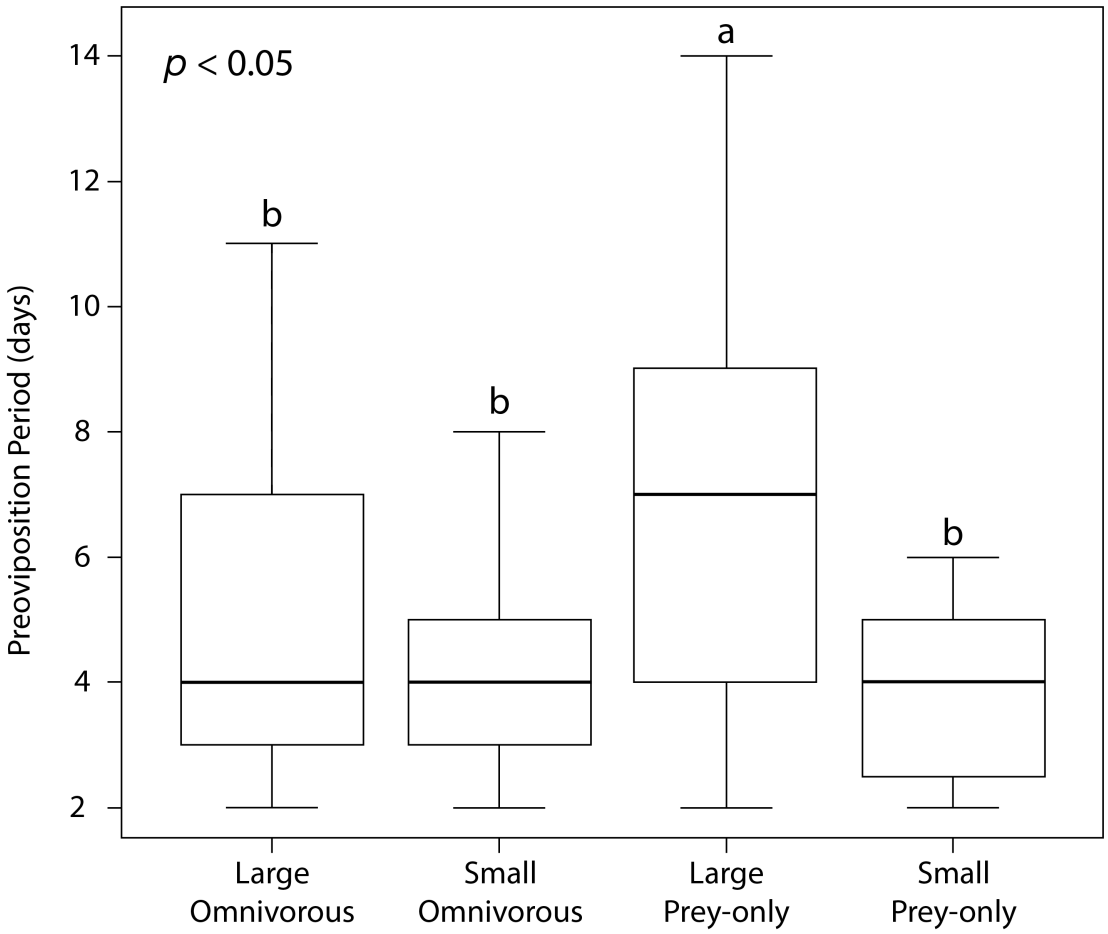

Figure 10 Median preoviposition period, with Interquartile ranges, of female H. convergens subjected to four different diet and size treatments (Large omnivorous = unrestricted larval food access, omnivorous adult diet - pollen, honey, greenbugs, moth eggs, Small omnivorous = 3hr daily larval food access, omnivorous adult diet - pollen, honey, greenbugs, moth eggs, Large prey-only = unrestricted larval food access prey-only adult diet - greenbugs, moth eggs, and Small prey-only = 3hr daily larval food access, prey-only adult diet - greenbugs, moth eggs) Treatments bearing the same letter were not significantly different (Tukey's test, $\alpha = 0.05$)

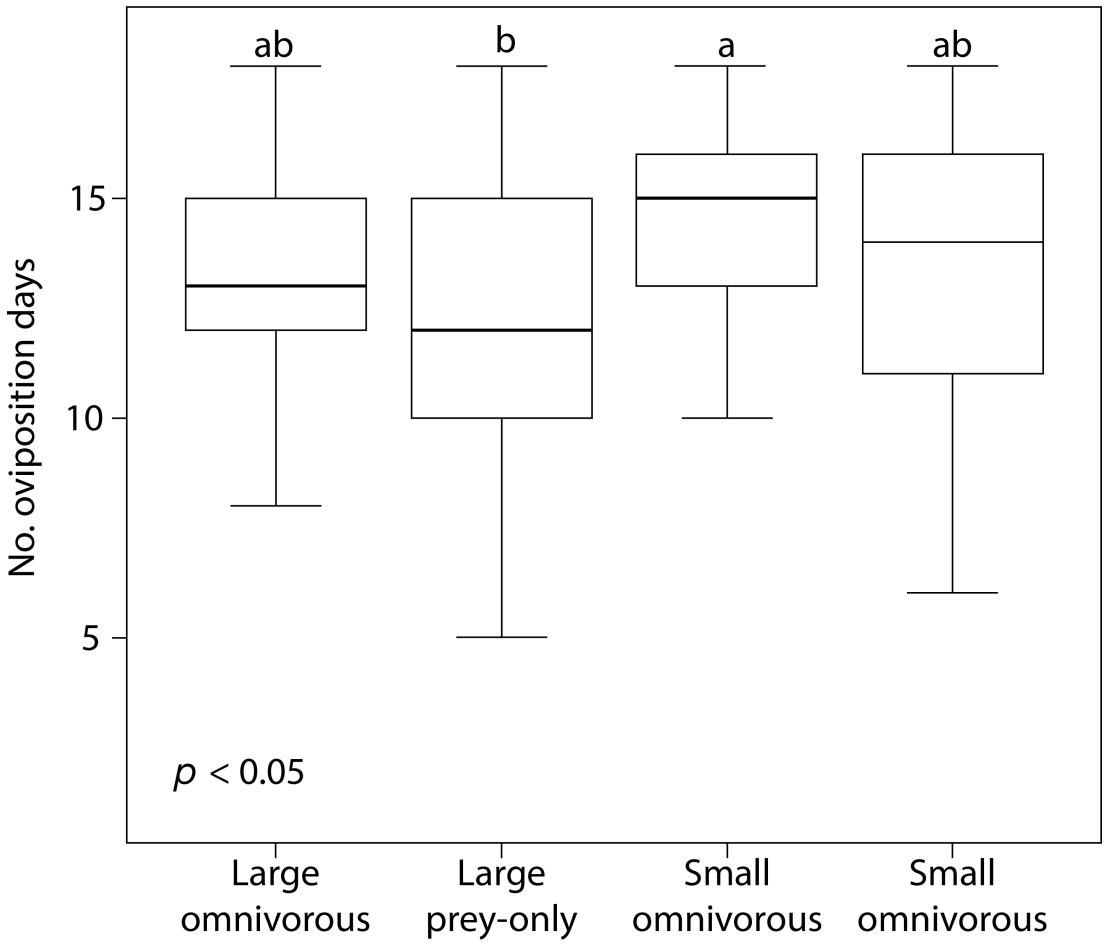

Figure 11 Median oviposition days, with interquartile range, of female H. convergens subjected to four different diet and size treatments (Large omnivorous = unrestricted larval food access, omnivorous adult diet - pollen, honey, greenbugs, moth eggs, Small omnivorous = 3hr daily larval food access, omnivorous adult diet - pollen, honey, greenbugs, moth eggs, Large prey-only = unrestricted larval food access prey-only adult diet - greenbugs, moth eggs, and Small prey-only = 3hr daily larval food access, prey-only adult diet - greenbugs, moth eggs) Treatments bearing the same letter were not significantly different (Tukey's test, $\alpha = 0.05$)

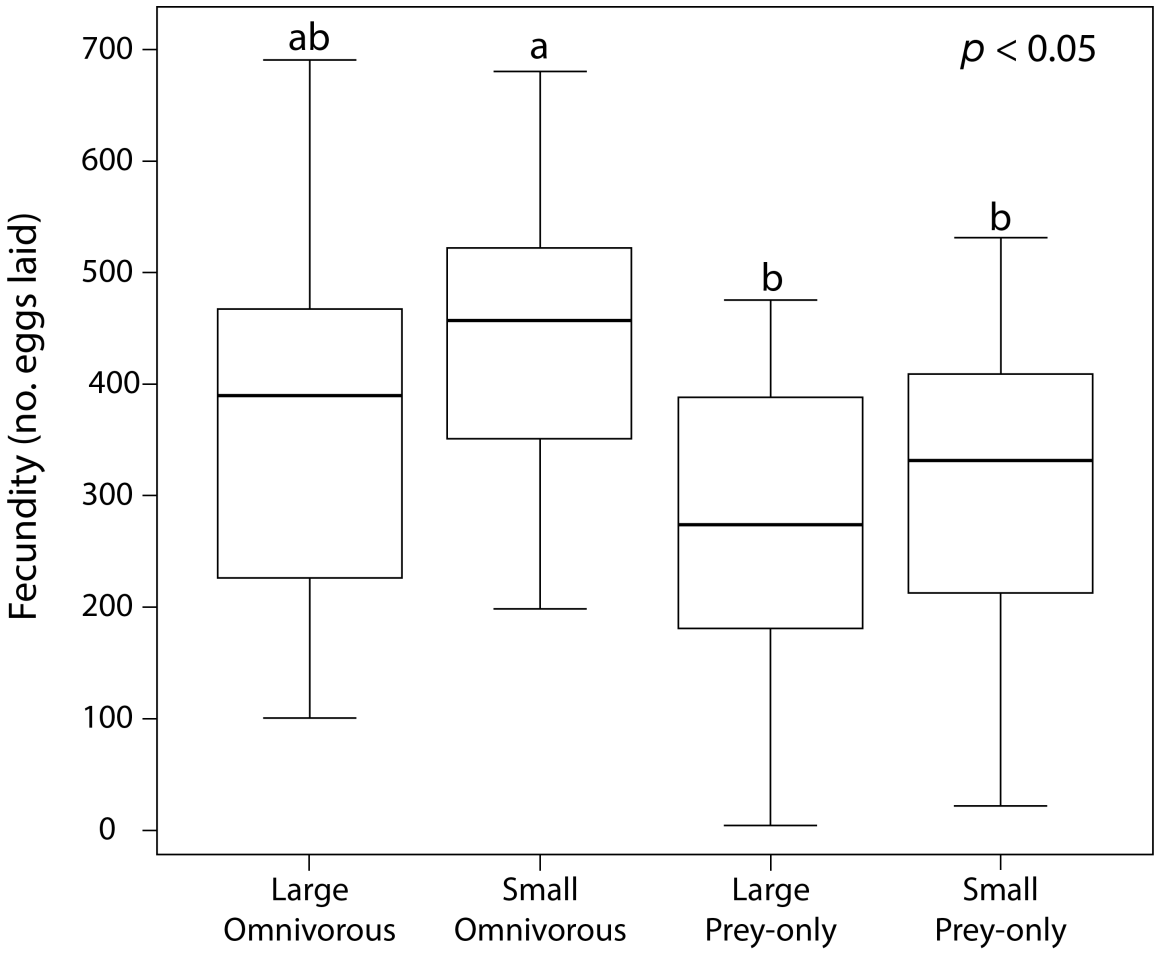

Figure 12 Median 18-d fecundities, with interquartile range, of female H. convergens subjected to four different diet and size treatments (Large omnivorous = unrestricted larval food access, omnivorous adult diet - pollen, honey, greenbugs, moth eggs, Small omnivorous = 3hr daily larval food access, omnivorous adult diet - pollen, honey, greenbugs, moth eggs, Large prey-only = unrestricted larval food access prey-only adult diet - greenbugs, moth eggs, and Small prey-only = 3hr daily larval food access, prey-only adult diet - greenbugs, moth eggs) Treatments bearing the same letter were not significantly different (Tukey's test, $\alpha = 0.05$)

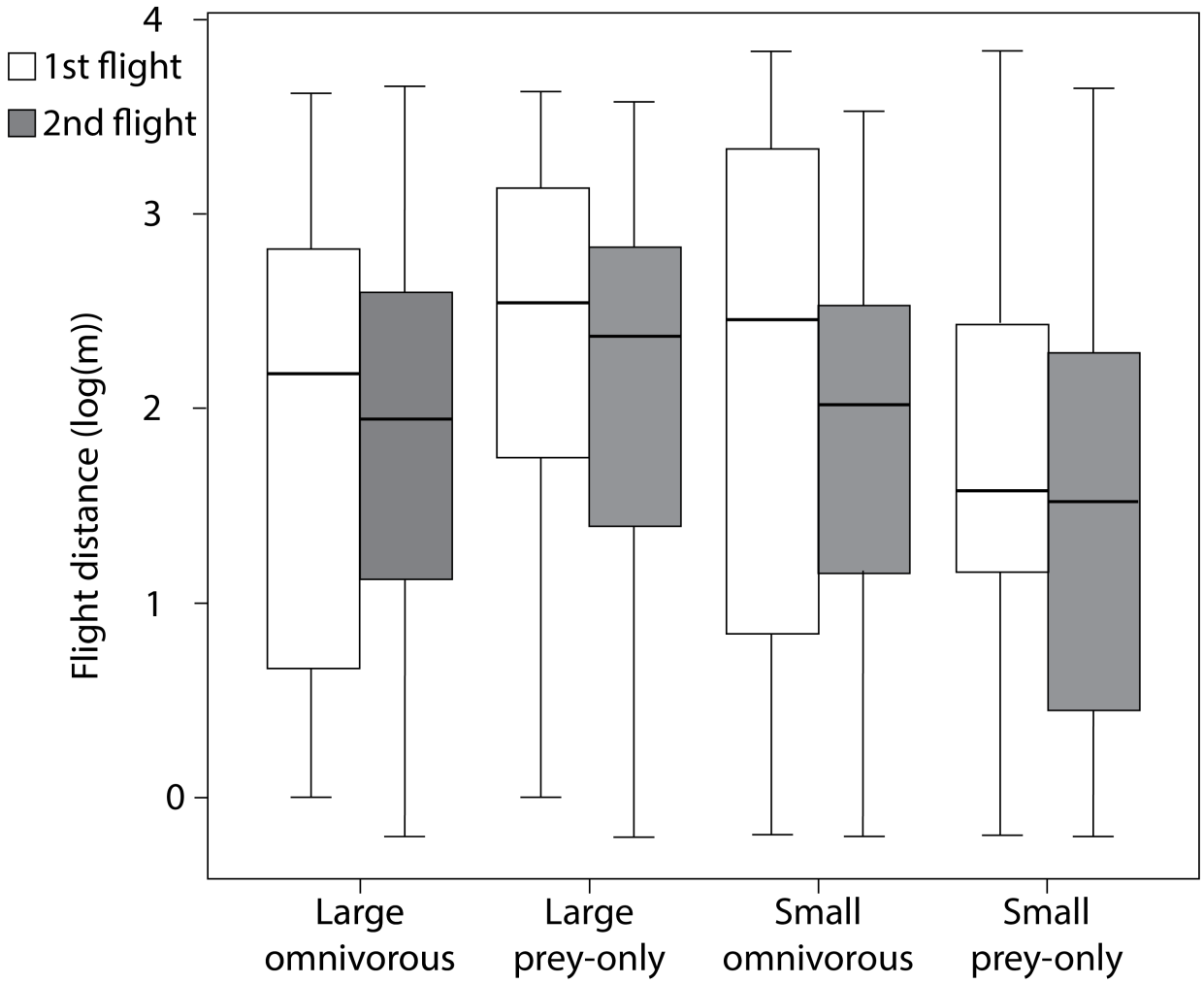

Figure 13 Median flight distance, with interquartile range, of female H. convergens subjected to four different diet and size treatments (Large omnivorous = unrestricted larval food access, omnivorous adult diet - pollen, honey, greenbugs, moth eggs, Small omnivorous = 3hr daily larval food access, omnivorous adult diet - pollen, honey, greenbugs, moth eggs, Large prey-only = unrestricted larval food access prey-only adult diet - greenbugs, moth eggs, and Small prey-only = 3hr daily larval food access, prey-only adult diet - greenbugs, moth eggs). White boxes indicate 1st flight, grey indicate 2nd flight

Neither adult body size (F = 0.001, df = 1,95, p = 0.975) nor adult diet (F = 0.17, df = 1,95, P = 0.685), affected the initial (pre-reproductive) flight distance of prereproductive females, and the interaction term between these factors was marginally significant (F = 3.72; df = 1,95; P = 0.057). Similarly, the distance flown by females after an 18-d reproductive period was unaffected by adult body size (F = 2.12, df = 1,95; P = 0.815) or adult diet (F = 0.055; df = 1,95; P = 0.148;

Figure 13). Total flight distance was positively correlated with longer preoviposition period (r^2 = 0.03, p = 0.044, Figure 14), suggesting a possible connection between oviposition hesitancy and greater dispersal effort. Female flight activity varied greatly, with total distance flown ranging from <5m to >7km. Beetles flew significantly further on their first flight test (mean ± SE = 785.2 ± 132.4 m) than on their second test (mean ± SE = 493.3 ± 83.2 m, t = 5.93; df = 1,98; P < 0.001). Regression analysis revealed that preoviposition period was negatively correlated with both number of oviposition days (F = 45.35; df = 1,97; P < 0.0001; r^2 = 0.32) and 18-d fecundity (F = 29.38; df = 1,97; P < 0.0001; r^2 = 0.02). However, fecundity was not correlated with flight distance, whether pre-reproductive (F = 0.11, df = 1,97; P = 0.741) or post-reproductive (F = 0.19, df = 1,97; P = 0.277).

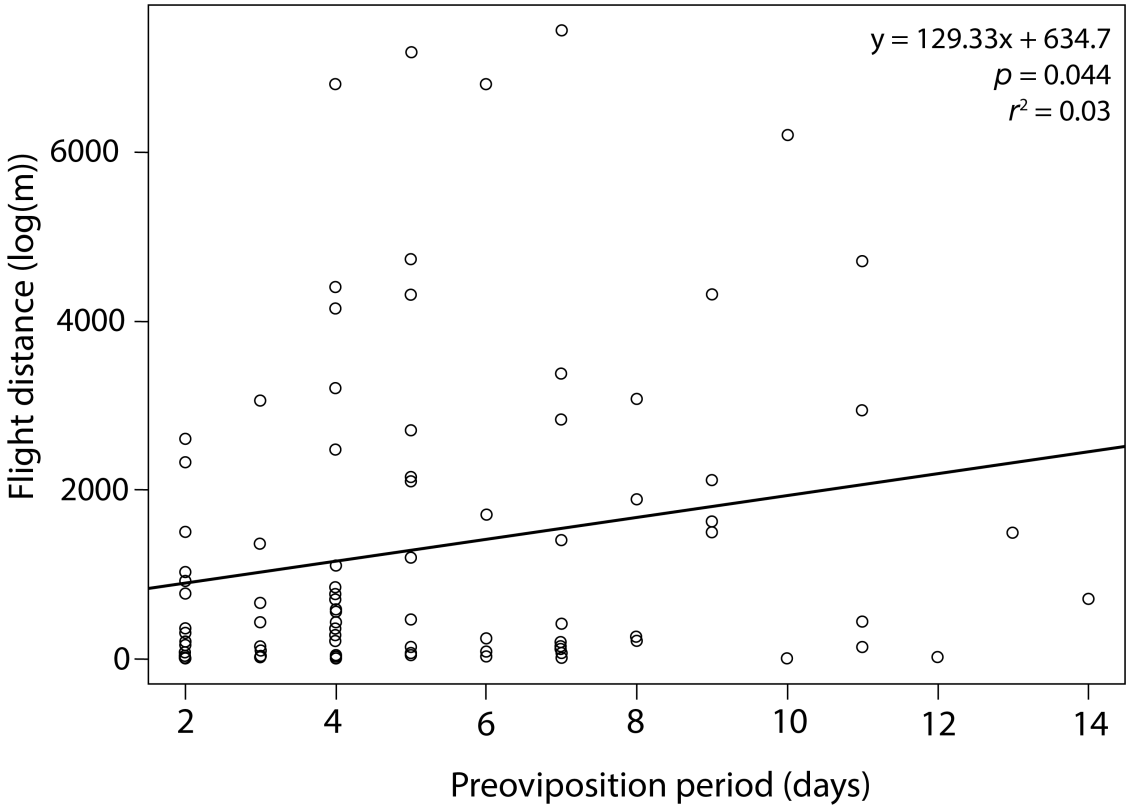

Figure 14 Linear regression of cumulative total flight distance covered by *H. convergens* females on their preoviposition period. equation: y=129.33x + 634.7

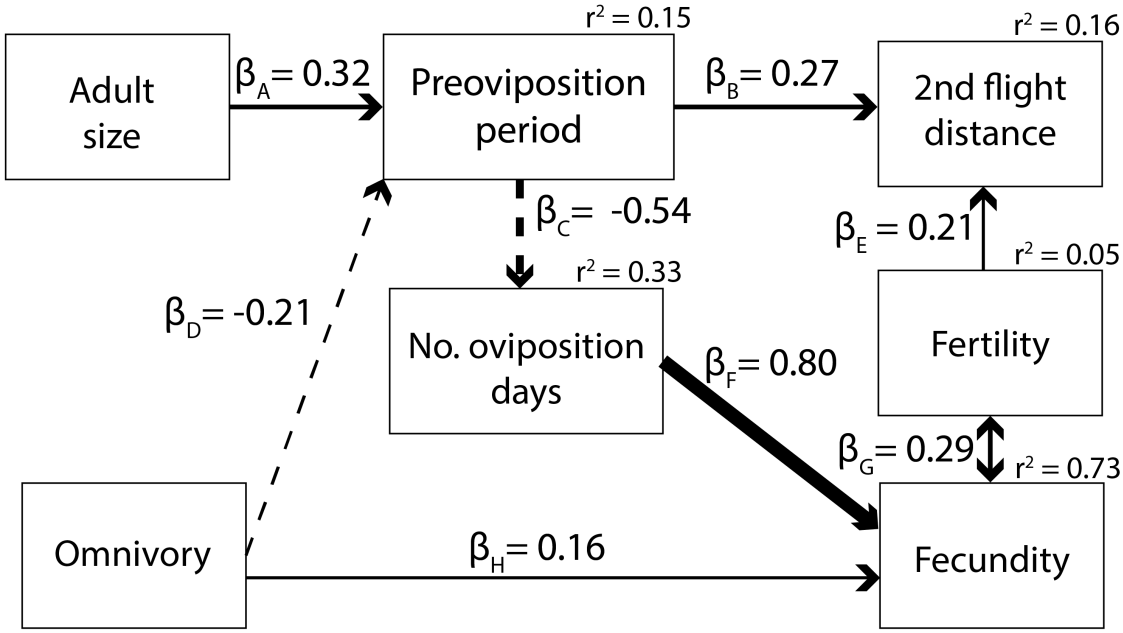

Figure 15 Structural equation model (SEM) of the effects of size and diet treatments on flight performance and reproduction of female H. convergens. Solid arrow represent positive causal relationships and dashed arrows, negative causal relationships. Arrows are scaled with the standard estimate (β) value to represent relative size of the effect. Coefficient of determinations (r^2) values indicate the proportion of the variation explained by the model. Fecundity is the number of eggs laid per female in 18 days of oviposition. Preoviposition period is the number of days from provision of the reproductive diet (greenbugs) to first oviposition. No. oviposition days is the number of days on which clutches were laid in the 18 day observation period. Flight distance is the total distance flown (in meters) during a 3 hour period on a tethered flight mill. Model fit: AIC = 56.5, df = 22, *p* = 0.98.

Structural equation modeling indicated no significant effect of pre-reproductive flight on subsequent reproductive success or flight behavior (Figure 15, Fisher's C = 10.5, df = 22, *P* = 0.98). Adult size and diet composition both influenced the preoviposition period, with large adult size delaying onset of oviposition (β_A = 0.32) and the omnivorous diet accelerating it (β_D = -0.21). Omnivory also had a direct positive effect on fecundity (β_H = 0.16) in addition to its indirect positive effects on fecundity via effects on preoviposition period (β_C = -0.54) and number of oviposition days, the latter having a direct positive effect on fecundity (β_F = 0.80).

Delayed onset of oviposition was also associated with greater post-reproductive flight distance ($\beta_B = 0.27$). Fertility and fecundity were positively correlated with one another ($\beta_G = 0.29$) and fertility was positively correlated with post-reproductive flight distance ($\beta_E = 0.21$).

When dependent variables are examined outside of the SEM, we see that both the number of oviposition days ($r^2 = 0.32$, $p < 0.0001$, Figure 16) and, to a lesser extent, total individual fecundity ($r^2 = 0.02$, $p < 0.0001$, Figure 17) were positively correlated with greater preoviposition period.

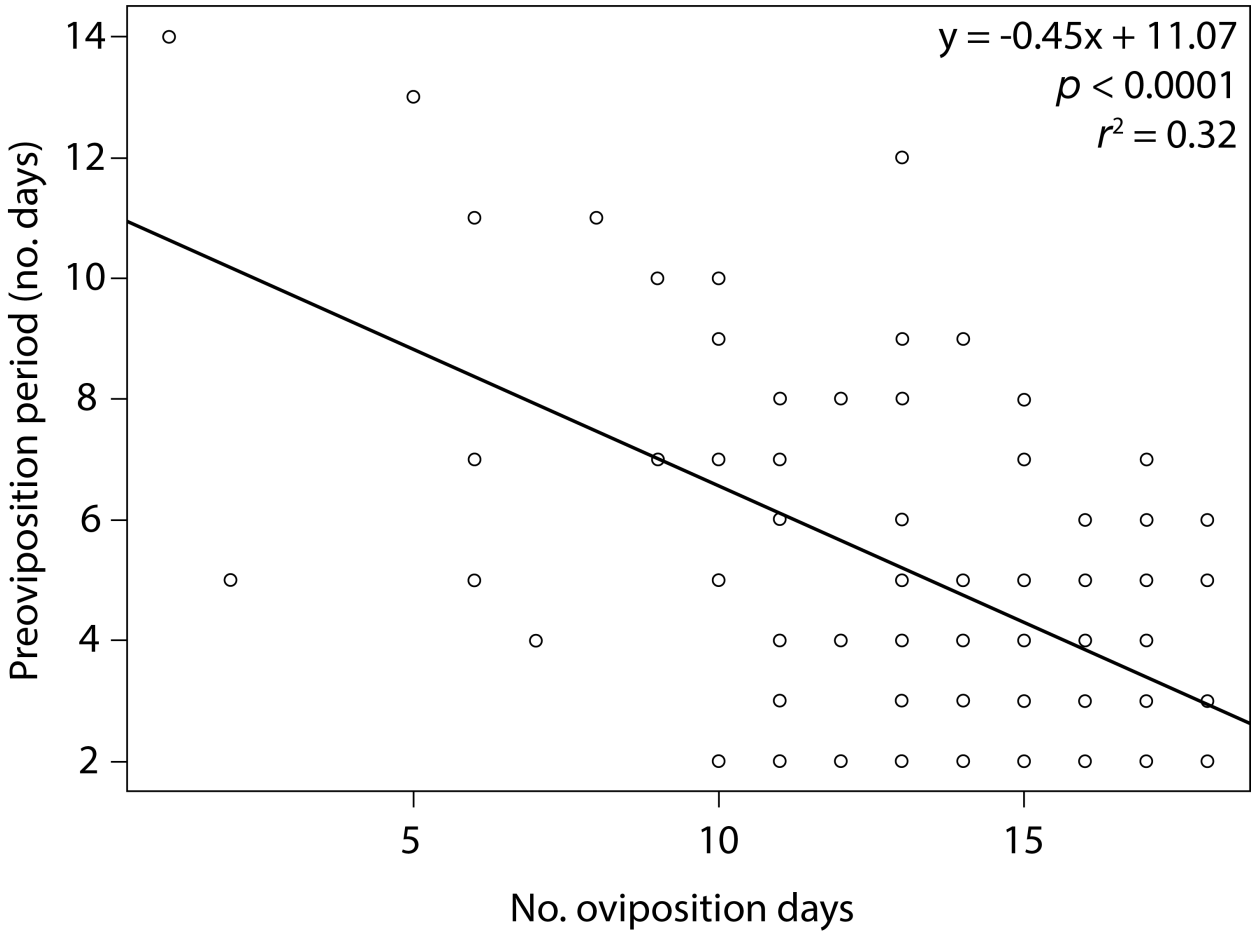

Figure 16 Linear regression of preoviposition period for *H. convergens* females on their oviposition days. equation: y=-0.45x + 11.07

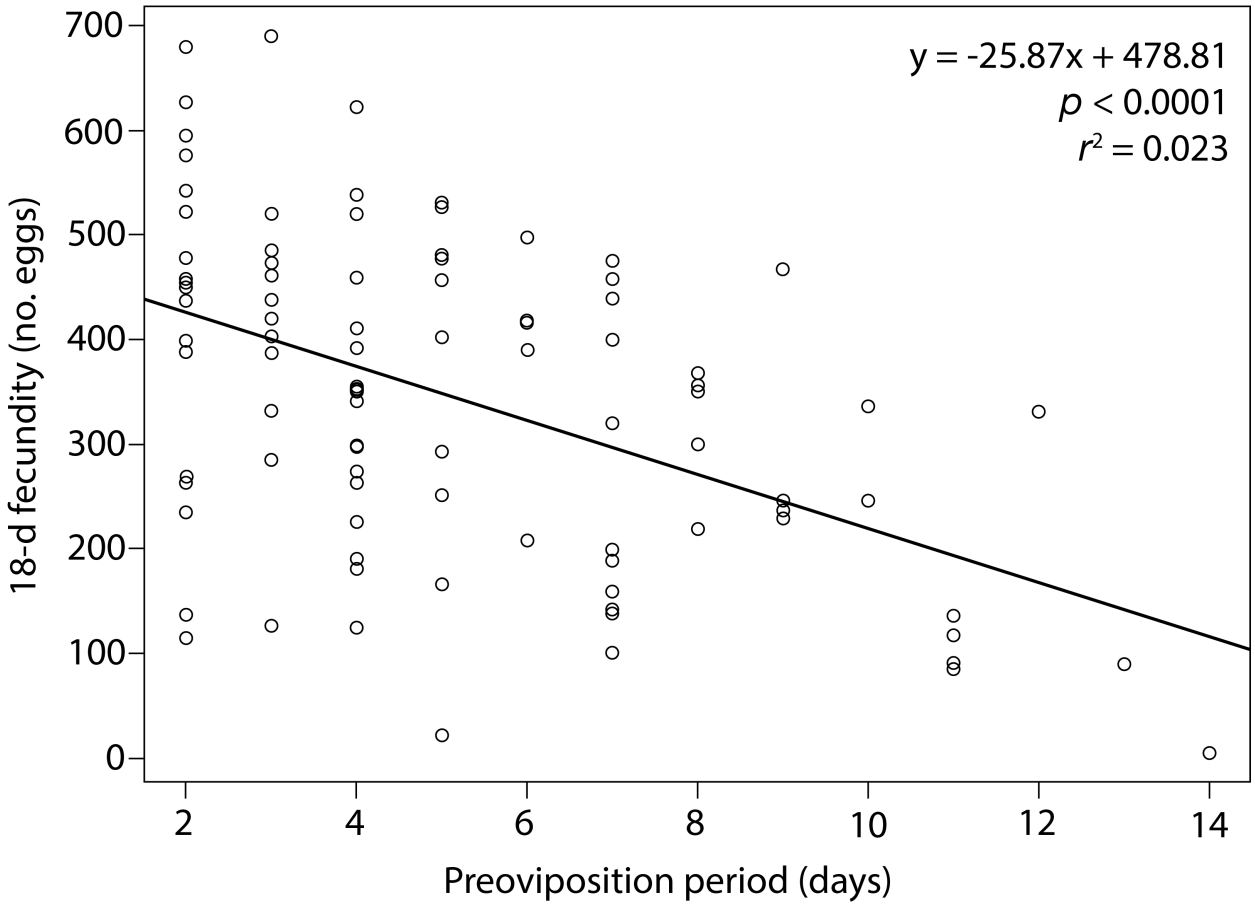

Figure 17 Linear regression of 18-d fecundity of *H. convergens* females on their preoviposition period. equation: y=-25.87x + 478.81

3.5 | DISCUSSION

The omnivorous adult diet resulted in greater female fecundity than did the prey-only diet, supporting our initial hypothesis, and confirming that floral resources complement the nutrition provided by prey, and are essential to maximal reproductive success in this species. Previous studies have similarly concluded that plant-derived foods generally enhance the performance of predatory coccinellids relative to prey-only diets (Lundgren, 2009), including *H. convergens* (Stowe et al., 2021). Supplemental (plant-derived) foods have long been recognized for their importance to coccinellid survival during periods of prey scarcity (Hodek 1996; Michaud and Qureshi, 2005), but may also provide phytosterols, key requirements for

development and reproduction that insects are unable to synthesize endogenously (Levinson, 1962; Behmer and Nes, 2003; Pilorget et al., 2010). Furthermore, feeding on carbohydrates can increase lipid reserves, which can improve insect longevity and overwintering survival (He and Sigsgaard, 2019), and can be rapidly oxidized to provide energy for flight (Toprak, 2020; Wang et al., 2020). The benefits of omnivory likely occur independent of prey diversity in the diet, as animal and plant-derived foods typically supply key macronutrients in differing ratios (Raubenhiemer and Jones, 2006; Raubenhiemer et al., 2007). Macronutrient ratios have been shown to affect growth, development, and survival in phytophagous coccinellids (Wang et al., 2018) and other insect herbivores (Behmer, 2009), and arthropod predators often forage selectively to balance their intake of protein, lipids and carbohydrates (Mayntz et al., 2005; Jensen et al., 2012). For example, a prey-only diet will be high in protein, which can reduce net energy intake due to digestive costs, and alter activity levels (e.g., Koemel et al., 2019).

Our hypothesis that large body size would result in greater female fecundity was not supported, seemingly contradicting previous work that examined the effect of *H. convergens* body size on fecundity over longer time frames (Vargas et al., 2012a; 2012b), although plant resources were not provided in those experiments. Adult size only had a marginal effect on fecundity within the 18-d observation period, and in the opposite direction of that expected, being highest for small females. This result may reflect fewer days of oviposition by LO and LP pairs within the period of observation, combined with delayed onset of oviposition in LP pairs. Oviposition days were strongly correlated with fecundity in the SEM model, and previous work has shown that daily fecundity peaks later in large *H. convergens* than in smaller ones (Vargas et al., 2012a), so a longer period of observation could reveal different body size effects. Fertility was unaffected by either diet or adult size, contradicting our hypothesis that larger body size

would benefit fertility. Once again, this result likely reflects the limited time frame of our observations. Maternal body size has no discernable effect on *H. convergens* egg fertility early in reproductive life, but its effects become more pronounced with advancing age, as fertility declines faster in both large and small females than in those of intermediate size (Vargas et al., 2012a).

Our hypothesis that access to floral resources would positively affect reproductive success was supported. Large beetles fed a prey-only diet experienced a delayed onset of oviposition relative to those receiving the omnivorous diet, suggesting that nutrients provided by floral resources enabled beetles to begin maturing eggs sooner when body size was large. However, the large beetles we produced are likely uncommon in nature, where food-limitation is the norm for most larvae at some point in development. If reproductive traits have evolved to be adaptive for the average body size, a large body size may impose some costs. Large individuals may have higher maintenance requirements than small ones, such that the soma of large beetles may (initially) take precedence over the gonads when key nutrients become limiting, as likely occurred in the prey-only treatment.

Despite the fact that body size and adult diet affected beetle reproductive parameters, neither treatment generated any significant variation in the distance flown by females on flight mills, either in pre- or post-reproductive tests. In large part, this was due to immense variation among females in total flight activity, which ranged from < 5 m to > 7km. Rankin and Rankin (1980) similarly reported large variation in distances flown by tethered *H. convergens* beetles, with 90% of individuals stopping after 30 min, while the remaining 10% were still flying after 12 h. We infer that *H. convergens* populations consist of individuals that vary greatly in intrinsic dispersal capacity, and that additional variation was contributed by differences in physiological

state, despite our best efforts to standardize beetle age and rearing conditions. Maes et al. (2014) showed that body size was positively correlated with greater flight distance in both *Harmonia axyridis* Pallas and *Cryptolaemus montrouzieri* Mulsant, although not in *Adalia bipunctata* (L.), so it is possible our observation period was not sufficiently long to resolve a body size effect. Although sugars, usually obtained from floral nectar, are the primary fuel for flight in many insects, including Diptera (Dunn et al., 2020), Hymenoptera (Suarez et al., 2005), and Lepidoptera (Su et al., 2021), our results indicate that *H. convergens* flight behavior does not depend on a source of simple carbohydrates in the adult diet. Other energy sources, most likely stored lipids, appear to support long distance flight in these beetles, as they do in many other insects (Arrese and Soulages, 2005; Toprak, 2020). This would be adaptive, given that *H. convergens* often need to make long distance migratory flights at times when floral resources may be unavailable, such as after emergence from hibernation or aestivation (Hagen, 1962).

Dispersal is energetically costly and is expected to reduce the energy available for subsequent foraging behavior and/or reproduction (Stearns, 1992).The SEM results revealed direct, but weak, relationships between post-reproductive flight distance and both length of the preoviposition period and egg fertility, implying that both delayed onset of oviposition and higher egg fertility were associated with greater flight activity in gravid females. More significantly, the shorter distances flown by females post-reproduction suggest that egg maturation depleted the energy reserves available for flight. This result stands in contrast to the findings of Steward et al. (1994) for the tarnished plant bug, *Lygus lineolaris*; which found that older bugs flew greater distances than prereproductive ones. Apparently, our adult diet treatments did not influence energy reserves sufficiently to affect flight activity within a 3 h period, nor did this period of flight deplete energy reserves sufficiently to negatively affect

subsequent reproductive capacity. These results are consistent with a long-range dispersal capability in this species, with much longer periods of flight required before energetic demands become significant.

Apparent trade-offs between flight ability (or energetic expenditure on flight) and reproductive effort have been reported in many insects (Zera and Harshman, 2001). For example, Guerra and Pollack (2007) showed that flight ability was negatively correlated with male courtship singing in the cricket *Gryllus texensis*. Roff (1986) compared 22 species of wing-dimorphic insects and found that brachypterous female morphs were more fecund and had earlier onset of reproduction than macropterous morphs, and Zera and Denno (1997) showed that flight capability carries reproductive costs for males as well as females. In the present study, we found that post-reproductive flight distance was positively correlated with preoviposition period in the SEM model, the latter being negatively correlated with number of oviposition days, and thus with fecundity, results that are consistent with trade-offs between reproductive effort and energy expenditure on flight.

The quality of the natal patch is known to have strong carryover effects on dispersal capacity. For example, high emigration rates of the backswimmer *Notonecta undulata* are associated with development in patches of high quality (Baines and McCauley, 2018). Because the quality of the developmental environment has the potential to shape adult phenotypes, it can have cascading effects on population dynamics and community structure that extend to landscape scales (Van Allen and Rudolf, 2013; 2016). Carryover effects of natal patch quality on body size and life history parameters will have significant implications for *H. convergens* population dynamics and the predation services this species provides in the agricultural landscape, as successive generations colonize different crops that vary in the quality of resources they provide.

Thus, the provision of floral resources either within, or adjacent to, crop fields will help sustain the vigor of *H. convergens* populations, and the efficacy of their biological control services. These services extend beyond control of aphids to the consumption of many other pests in their early life stages, even though the latter may not support beetle reproduction (Michaud, 2018).

Our results here illustrate the importance of floral resources to the fitness of arthropod predators that provide biological control services as they migrate across the agricultural landscape. Broadleaf plants have distinct, usually brief, flowering seasons, so a diversity of species that vary in flowering phenology will be required to ensure these resources are available over extended periods (Bianchi et al., 2006).The clear benefits of plant derived resources in the diet of *H. convergens* include increased fecundity, earlier oviposition and higher numbers of oviposition days, Similarly, the relatively unexplored flight behavior of *H. convergens* may have significant implications for migration, emigration, and foraging flight in this species. Many cultural practices have the potential to support, or disadvantage, populations of coccinellids and other generalist predators in the landscape and warrant careful ecological assessment of their ecological impacts. For example, over-zealous control of all broadleaf plants on roadsides and marginal lands constitute an unnecessary reduction of potential floral resources in service of cultural expectation alone, while providing no direct benefit to crop production. Also concerning is the increasingly wide-spread use of insecticidal seed treatments. This practice not only impacts predators negatively via prey deprivation during crop establishment, but also creates various routes of direct and indirect exposure for beneficial species, with both lethal and sublethal consequences (Seagraves and Lundgren, 2012; Gontijo et al., 2014; Moscardini et al., 2015; Bredeson and Lundgren, 2018; 2019). In contrast, farmer tolerance of flowering broadleaf plants in marginal and uncultivated areas, and the planting of cover crops as an alternative to sterile,

'chem-fallow' periods that create starvation conditions for all arthropods, represent feasible approaches to supplement valuable floral resources for predator populations as they move among crops in the agricultural landscape.

3.6 | REFERENCE

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Chapter 4 - Resource Amount and Access Period Influence Flight and Reproductive Behavior in *Hippodamia convergens* (Coleoptera: Coccinellidae).

4.1 | ABSTRACT

1. Industrial-scale agriculture creates a mosaic of large monocultures in the landscape, where seasonal cropping cycles generate discontinuous resource availability for insect predators both spatially and temporally. In this environment, selection will favor predator movement and reproduction behaviors that optimize location access and effective utilization of resource pulses that are both patchy and ephemeral in nature.
2. The convergent lady beetle is a highly mobile predator that provides a model system to study predator movement and reproduction within the crop landscape. We tested how discontinuous periods of food access that mimic fluctuating aphid populations and beetle tracking of seasonally available prey among crop fields, would influence flight behavior and reproduction and possibly modify any energetic trade-offs between these behaviors. Adult beetles were provided either short (3hr) or long (6hr) food pulses daily (continuous availability) or short (6h) or long (12h) food pulses every other day (discontinuous availability). Periods of tethered flight (3h) measured female flight activity before and after an 18 day reproductive period.
3. Discontinuous food access delayed onset of oviposition in the high food quantity treatment, fewer females laid eggs overall and 18 d fecundity was lower compared to continuous provision of the same food quantity. Longer preoviposition periods were associated with fewer reproductive days and lower fitness. Flight distance and fecundity

were negatively correlated, suggesting that energetic expenditure in flight can deplete energetic reserves otherwise used for subsequent reproduction.

4. The negative fitness effects of discontinuous resource access at fine temporal scales reveal how gaps in resource availability can potentially impact lady beetle population dynamics and their ecosystem services within the agricultural landscape. Understanding how resource availability patterns affect lady beetle fitness and behavior can inform strategies for agricultural land management that enhance conservation biological control.

4.1.1 Keywords

dispersal, energetic tradeoffs, resource discontinuity, biological control, insect predators, ecological tradeoffs, flight capacity

4.2 | INTRODUCTION

The negative effects of disturbance and resource fragmentation on insect biodiversity and ecosystem functions in agricultural landscapes are well documented (Samways 1989, Bianchi et al., 2009; Chaplin-Kramer et al., 2011; Tscharntke et al., 2012; Angeler 2016; Haan et al., 2020). Disturbances due to tillage, harvest, and chemical inputs can lead to direct insect mortality (Pearsons & Tooker, 2017; Tooker et al., 2020; van der Meer et al., 2020) or reduce habitat and food resources necessary for insect survival (Raven & Wagner, 2021; Wagner 2021), thus decreasing ecosystem services such as biological control and pollination. Previous studies have examined the relationship between resource amount and environmental disturbance (Ojima et al., 1994; Solbrek, 1995; Karakoc et al., 2018; Wagle & Gowda, 2018), but less information exists concerning the effects of variable access to resources, i.e., resource discontinuity (Welch & Harwood, 2014; Schellhorn et al., 2015; Spiesman et al., 2020). Resource discontinuities created by disturbances can be spatial (i.e., rich resource patches are physically separated within resource matrices comprised largely of low resource regions) and/or temporal (i.e. rich resource patches are only ephemerally available) creating a landscape of unreliable resource availability in space and time. Resource discontinuity is likely to increase under climate change, as more frequent extreme weather events such as heat waves, droughts, and heavy precipitation events (Folland et al., 2002; Thornton et al 2014) alter resource availability and stress insect communities, potentially diminishing the ecosystem services that they provide.

Insects that can persist within spatially and temporally variable resource environments will face tradeoffs between fitness-critical behaviors such as dispersal and reproduction. The ability of predators to track, or even anticipate, prey populations within annual crops can

determine their survival and the effectiveness of their ecosystem services (Wissinger, 1997; Landis et al., 2000; Prasifka 2004). For example, mobile predators may relocate to exploit alternative resources when the availability of a primary resource fluctuates capriciously (Wissinger, 1997; Landis et al., 2000), potentially incurring energetic tradeoffs with other life history traits such as reproduction. Therefore, high mobility may be a prerequisite for success when generalist insect predators colonize agricultural environments, at least over short time frames (Wissinger, 1997; Xiao et al., 2017). However, flight is energetically costly, so excessive flight activity driven by food supply disruptions may diminish predator fitness and the biological control services they provide. Reproduction is also energy-intensive, and the allocation of energetic 'capital' to dispersal may reduce the amount that can be allocated to reproduction in the future (Stearns, 1992). Considering these energetic tradeoffs, any cultural interventions that minimize resource variability within agricultural landscapes, i.e., the use of cover crops, intercropping, or field crop complementarity, could reduce the energetic burden of local migrations for predators, leaving them with greater reserves to devote to foraging and reproduction when prey patches are finally encountered.

The present study addressed whether resource quantity and temporal availability would influence flight capacity and reproductive success in a highly mobile insect predator, the convergent lady beetle, *Hippodamia convergens.* The convergent lady beetle is one of the most abundant species of coccinellid on the High Plains where it is a key biocontrol agent of important cereal aphid pests such as greenbug, *Schizaphis graminum* Rondani (Rice & Wilde, 1988), Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Nechols & Harvey ,1998), and sugarcane aphid, *Melanaphis sacharri* Zehntner (Colares et al., 2015). Although *H. convergens* can be considered a specialized aphid predator, as it relies heavily on aphids for reproduction (Michaud

& Qureshi, 2006), both larvae and adults survive periods of aphid scarcity by exploiting a wide range of supplementary foods that include the immature stages of other insects and various plant-derived resources (Lundgren, 2009, Mercer et al., 2020. Stowe et al., 2021). On the High Plains, these beetles typically produce their first generation in wheat or alfalfa with the resulting adults migrating to summer crops and switching among prey types in response to their availability, ultimately reproducing in different crops than those that supported their initial development (Tillman & Cottrell, 2012, Bastola et al., 2016). Spatial and temporal disturbances inherent to large-scale farming operations can increase energy demands for *H. convergens* adults by increasing the need for dispersal by flight. *Hippodamia convergens* are strong flyers (Hagen, 1962; Jeffries et al., 2013) and will emigrate from early season crops to later season crops even before the latter have developed large prey populations (Prasifka, 2004). Therefore, this species has evolved specific adaptations to exploit seasonal patterns of changing resource availability across agricultural landscapes. However, these seasonal patterns of dispersal likely represent sizable energetic costs for the migrant adults that may manifest as currently unmeasured tradeoffs in reproductive success.

We hypothesized that *H. convergens* adults fed larger quantities of food would fly greater distances and achieve higher reproductive success than those fed lower amounts, without significant effects on egg fertility nor timing of oviposition. Likewise, we hypothesized that sporadic (discontinuous) access to food would reduce flight distance, and female fecundity, and delay onset of oviposition relative to more continuous access. We also hypothesized that the overall quantity of food would interact with its pattern of availability, with lower quantities resulting in more severe negative effects when availability is discontinuous. We also hypothesized that discontinuous resource availability would result in greater initial flight

distance in the low food quantity treatments, assuming that less desirable conditions would incentivize emigration, with more continuous access to food having the opposite effect. Though we expect that low food treatments in general will support shorter flight distance than high food treatments overall. Finally, because of the many ways that food amount and continuity could directly and indirectly affect reproduction and behavior, we used a structural equation model (SEM) framework (Figure 18), to disentangle how resource amount and continuity of access would affect the presumed energetic tradeoff between flight distance and reproduction.

4.3 | MATERIALS AND METHODS

4.3.1 | Insect colony

Adult *H. convergens* beetles were collected from wheat fields at the Kansas State University Agricultural Research Center in Hays, Kansas (38°51'32.1"N 99°20'07.7"W) in June of 2020. Beetles (ca. 150) were placed in a 1L glass mason jar covered with muslin netting and held at 24 ± 1 °C 50 – 60% RH, with a photoperiod of 16:8 (L:D). Jars were filled with wax paper strips as harborage and provisioned with frozen eggs of *Ephestia kuehniella* every other day, with water provided on a cotton wick. Field-collected beetles are often parasitized by *Dinocampus coccinellae* Schrank (Hymenoptera: Braconidae), so the colony was inspected daily to remove any emerging wasps before they could complete pupation and emerge to attack other beetles in the colony.

A colony of greenbugs, *S. graminum*, was established from individuals collected from wheat at the same location as the beetle colony. The colony was reared on wheat seedlings grown in metal trays containing a soil/vermiculite/peat moss mixture (1/1/1). Trays were

germinated in the greenhouse and infested in growth chambers under fluorescent lighting at 24 ± 1 °C, 50 – 60% RH, and a 14:10 (L:D) daylength. Wheat seedlings were infested by clipping infested wheat leaves from declining trays and distributing them across a new tray of seedlings at the two-leaf stage.

4.3.2 | Experimental Insects

Maternal females (n=12) were removed from the stock colony and isolated in plastic petri dishes (5.5 cm diam.) where they were provided greenbugs *ad libitum* to induce oviposition. After the fertility of each female was confirmed by observing the eclosion of several clutches, two successive days of oviposition were collected from each female with the first clutches held at 18 ± 1 °C, 50-60%, and 14:10 (L:D) for 24h to slow eclosion. Eclosing neonates were allowed to disperse naturally from their egg clusters before they were isolated in petri dishes (as above). This gave teneral larvae a chance to harden and consume their chorions, as occurs naturally. The maternal ID of each larvae was tracked so that similar numbers of siblings could be assigned to each treatment and siblings would not be paired for reproduction.

All neonate larvae were reared under the same environmental conditions as the adult colony with *ad libitum* access to frozen *E. kuehniella* eggs and water provided in a small square of sponge, both refreshed daily. Once larvae pupated, any remaining food was removed from the dishes and pupae were monitored daily for emergence. All adults were sexed and weighed on an analytical balance (Mettler Toledo, AG285, Columbus, OH) within 24 hours of emergence. Pairs of non-sibling adults were established in ventilated snap-cap plexiglass vials (4.9 cm diam × 9 cm ht) where they were allowed to mature and mate for 12 days. During this period, they were provisioned with frozen *E. kuehniella* eggs (ca. 5mg), fresh foliage of wheat

seedlings, pulverized bee pollen, diluted honey (1:2 honey:water) on a small cube of sponge, and water on a second sponge square. No aphids were provided during this maturation period to prevent oviposition prior to experimental procedures. All plant resources were refreshed daily, *E. kuehniella* eggs were provided every three days, water and diluted honey as required. This diet was designed to mimic patterns of food availability under field conditions when first generation adults emigrate from natal fields and enter reproductive diapause, prior to encountering aphid populations that would support their reproduction (Michaud & Qureshi, 2006).

4.3.3 | Assays of flight behavior

A series of flight mills were constructed, modified from the instructions provided by Attisano et al. (2015), which allowed beetles to fly in a 10 cm diameter circle. A small dot of magnetic paint (Rust-oleum magnetic primer) was placed on the right elytra of each female beetle using a toothpick. After allowing 24 hours for the paint to dry completely, each female could then be magnetically attached to the flight mill arm using a small neodymium magnet. Flight tests were completed in a climate controlled growth chamber set to 24 ± 1 ºC, 50 – 60% RH. Each beetle was attached to one end of a rotating arm with the opposite end passing through an infrared sensor to record the number and timing of revolutions. This information was collected via an Arduino MEGA 2560 REV3 single board microcontroller and a terminal program (CoolTerm) for data collection. After flying for a total of 3 hours each female was returned to their container with their mate and assigned to one of diet four food access treatments, as described below. Female beetles were flown once prior to reproduction, and once again after they completed the 18 day oviposition period, measured from their first day of oviposition, and the total distance flown by each female on both dates were recorded.

4.3.4 | Food access treatments and experimental design

The experiment was constructed as a 2×2 factorial design which varied resource amount (high vs. low) and resource access (continuous vs. variable) to quantify the effects of these two aspects of resource availability (quantity and period of access) on flight behavior and subsequent reproductive success. Mature lady beetle couples were randomly assigned to one of four food access treatments: (1) High amount, continuous availability, "HC" (access to 6h of ad libitum food daily), (2) Low amount, continuous availability, "LC" (access to 3h of ad libitum food daily), (3) High amount, discontinuous availability diet, "HD" (access to 12h of *ad libitum* food every other day), and (4) Low amount, discontinuous availability diet, "LD" (access to 6h of *ad* libitum food every other day). This assortment of treatment conditions allows us to disentangle the effects of resource amount and resource continuity. The diet comprised *S. graminum* aphids provided on excised wheat seedling leaves and frozen *E. kuehniella* eggs (ca. 5mg), both provided fresh at the start of each feeding period. Manipulation of available food biomass is challenging when aphids are used as prey; nymphal instars vary greatly in size and apterous adults continue to reproduce even when removed from host plants. Therefore, we opted to control periods of access to *ad libitum* food rather than trying to estimate the biomass of aphids or other food consumed, an approach that has been successfully used in previous work (e.g., Vargas et al., 2013).

4.3.5 | Reproduction

Following the 12-day maturation period, beetle pairs were supplied with greenbugs *ad libitum* on the excised leaves of their host plants to induce oocyte maturation in female, which

requires 3 – 4 days (Michaud & Qureshi, 2006). Couples were checked every day for oviposition and egg clutches collected by simply moving adult beetles to a clean snap cap vial. All egg clusters were held until eclosion under the same environmental conditions as the stock colony so that fecundity and egg fertility could be recorded for each beetle pair. Males were held together with females throughout the observation period to ensure female fertility was maintained. Reproduction was recorded for 18 days for each couple, counted from the first oviposition day, a period long enough to capture any effect of the treatment on reproductive success. Females that failed to lay any eggs within 30 days after the inception of the reproductive diet were excluded from analysis of reproductive data.

4.3.6 | Statistical analysis

Assumption tests for equality of variance (Levene's test) and normality (Shapiro-Wilk test) were used evaluate all data. We analyzed treatment effects on preoviposition period, fecundity, egg fertility, total oviposition days, and flight distance using separate two-way ANOVAs followed by Tukey HSD for posthoc multiple comparisons. Main effects and interaction effects were investigated for the resources continuity of access variable and the resource amount variable. Oviposition days were tallied for each female as the number of days on which at least one egg was laid. Clutches were considered the total number of eggs laid by each female in one day. Fecundity was tallied as the total number of eggs laid, and egg fertility as the percentage of eggs hatching. Preoviposition period was calculated for each female as the number of days from provision of the reproductive diet (i.e. *S. graminum* provided ad libitum) until the first clutch was laid. Flight distance was calculated by counting the number of flight mill revolutions and multiplying by revolution circumference.

To examine the direct and indirect relationships between feeding treatment and fitness metrics (preoviposition period, fecundity, egg fertility, oviposition days), and flight, we used a structural equation modeling (SEM) framework. Experimental treatments were coded as 0 (low food quantity) and 1 (high food quantity) and resource access as 0 (discontinuous) and 1 (continuous). We predicted direct relationships between food quantity / access discontinuity and both reproductive metrics and post-reproductive flight distance (Figure 18, paths A, C, D, H, K, M, O, P). We also wanted to characterize relationships between pre-reproductive flight distance, reproduction, and flight performance (paths B, I, N, L). Initial flight distance was included as a predictor in the SEM model selection process because the distribution of initial flight distances indicated intrinsic variation in flight activity among females. Finally, we were interested in relationships between fitness and flight (paths E, F, G, J, N, Q, R), specifically, whether tradeoffs existed between energy expended in flight effort and reproduction which could diminish the availability of energetic reserves for subsequent flight and/or reproductive effort.

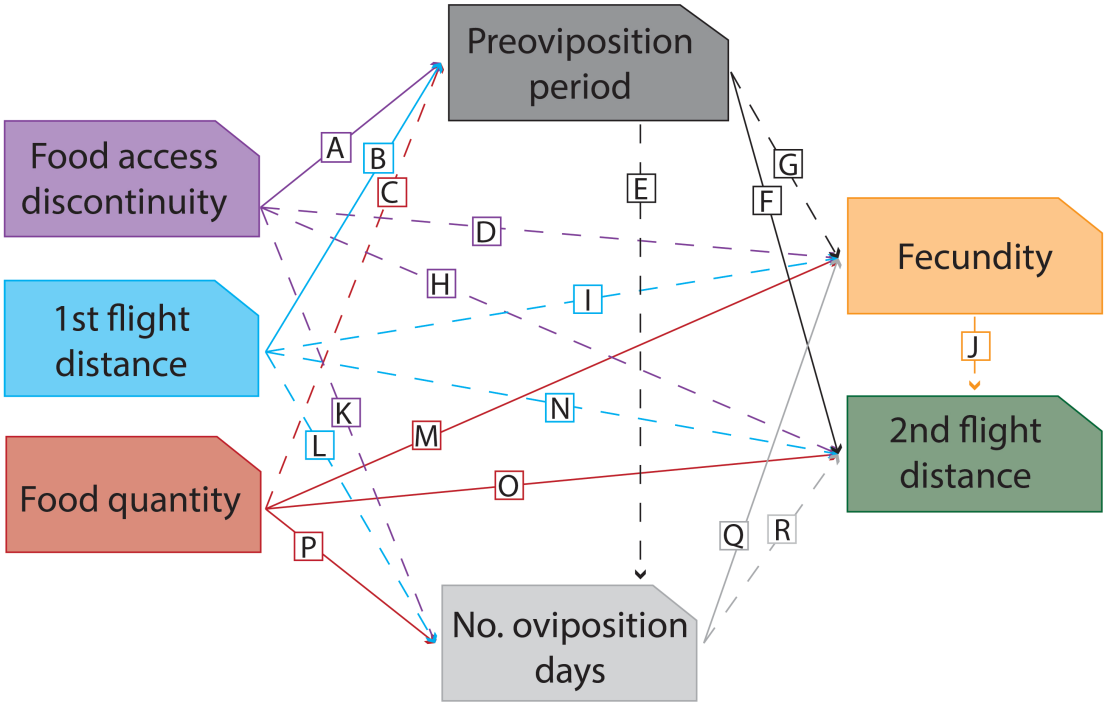

Figure 18 Hypothesis structural equation model: Higher food quantity is predicted to shorten preoviposition period and increase no. oviposition days, 18-d fecundity, and second flight distance. Discontinuity of food access and first flight distance are both predicted to lengthen the preoviposition period and reduce 18-d fecundity, no. oviposition days, and second flight distance. Longer preoviposition period is expected to decrease no. oviposition days and total fecundity and increase 2nd flight distance. Increased no. oviposition days is expected to increase fecundity and decrease 2nd flight distance.

Model selection was performed using maximum likelihood methods and linear relationships between variables were determined using bivariate scatterplots. We assessed overall goodness of fit using Akaike's Information Criterion (AIC) in a step-wise process of elimination. At each step, non-significant interactions were removed starting with the highest p-values in the coefficient list; where tests of directed separation were significant or marginally significant, predictors were returned to the model starting from the lowest available *p* value. At each step, AIC was consulted and only steps that reduced AIC were retained. For models with similar AIC values ($\Delta < 2$) the more parsimonious model was chosen (Grace, 2006; Burnham &

Anderson, 2002). All analyses were conducted in R version 4.0.3 using the piecewise SEM package (Lefcheck, 2016).

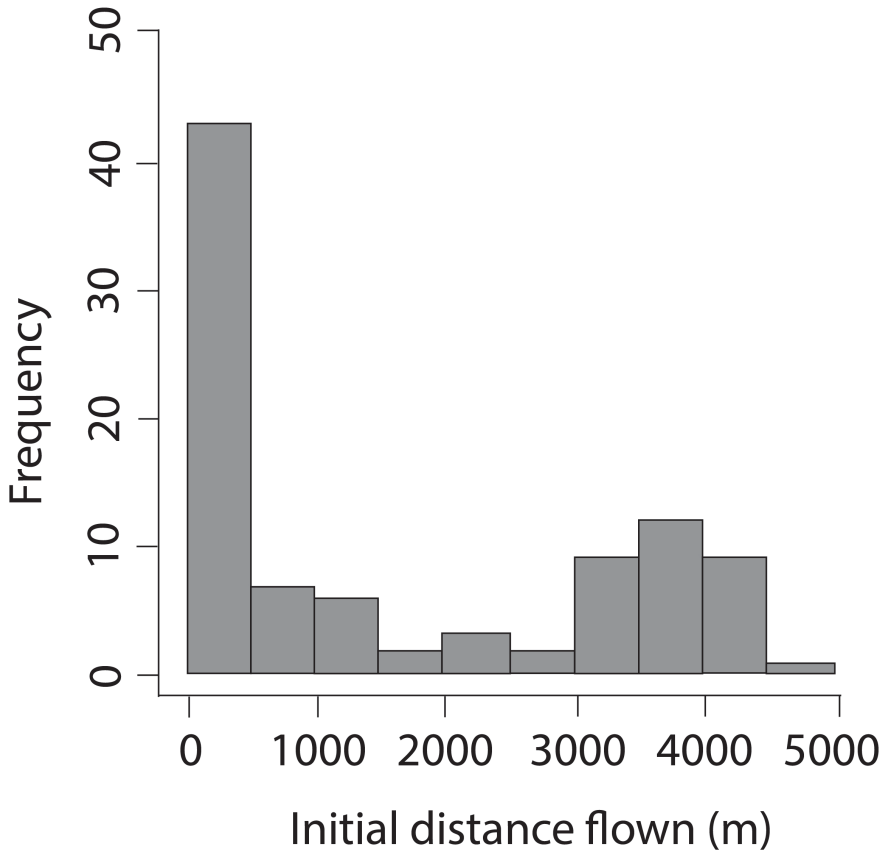

Figure 19 Frequency distribution of distances flown during first flights by pre-reproductive *H. convergens* females.

4.4 | RESULTS

A total of 300 larvae were reared to adulthood, with 12 deaths and 1 escape yielding 287 adults. Mean time from oviposition to adult emergence was 23.0 ± 1.0 days. Females had greater fresh weight at emergence than males (20.6 ± 2.6 mg vs.16.3 ± 1.8 mg, $F_{1, 285} = 273.1$, $p <$

0.005). Initial flight distances were measured before differential food access treatments were applied, and females showed a bimodal distribution (Figure 19). Whereas most females flew only a short distance, a subset exhibited greater flight propensity, flying between 1.5 and 2.5 km.

Fewer females broke diapause and became reproductive in the low food quantity / discontinuous access treatment than in any other treatment ($X^2 = 10.47$, df = 3, p = 0.015, Figure A). Among reproductive females, both food quantity ($F_{3,78} = 7.0$, $p = 0.016$) and access period ($F_{3,78} = 7.0$, $p < 0.001$) affected preoviposition period, with a significant interaction between these factors ($F_{3,78} = 7.0$, $p < 0.037$). The HC treatment produced the shortest preoviposition period, and the HD and LD treatments the longest, with the LC treatment intermediate and not significantly different from the others (Figure 20). Fecundity was significantly increased by continuity of food access ($F_{1,80} = 14.7$; $p < 0.001$) and food quantity ($F_{1,80} = 7.4$, p = 0.008, Figure 21), but these two factors did not interact ($F_{3,78} = 9.48$; $p < 0.001$). The HC treatment produced the highest fecundity, followed by the HD treatment, with the LD treatment yielding the lowest, and the LC treatment intermediate between HD and LD. Egg fertility was affected by food quantity ($F_{3,78} = 7.4$; p = 0.001) but not by continuity of food access ($F_{3,78} = 7.4$; p = 0.49), with lower food quantity reducing egg fertility relative to high (Figure 22). Post-reproductive flight distance was not significantly affected by food quantity ($F_{3,90} = 0.6$; p = 0.65) or continuity of food access ($F_{3,90} = 0.6$; p = 0.45), nor did the two factors interact significantly ($F_{3,90} = 0.6$; p = 0.74); mean post-reproductive flight distance was (1382.6 ± 168.5 m).

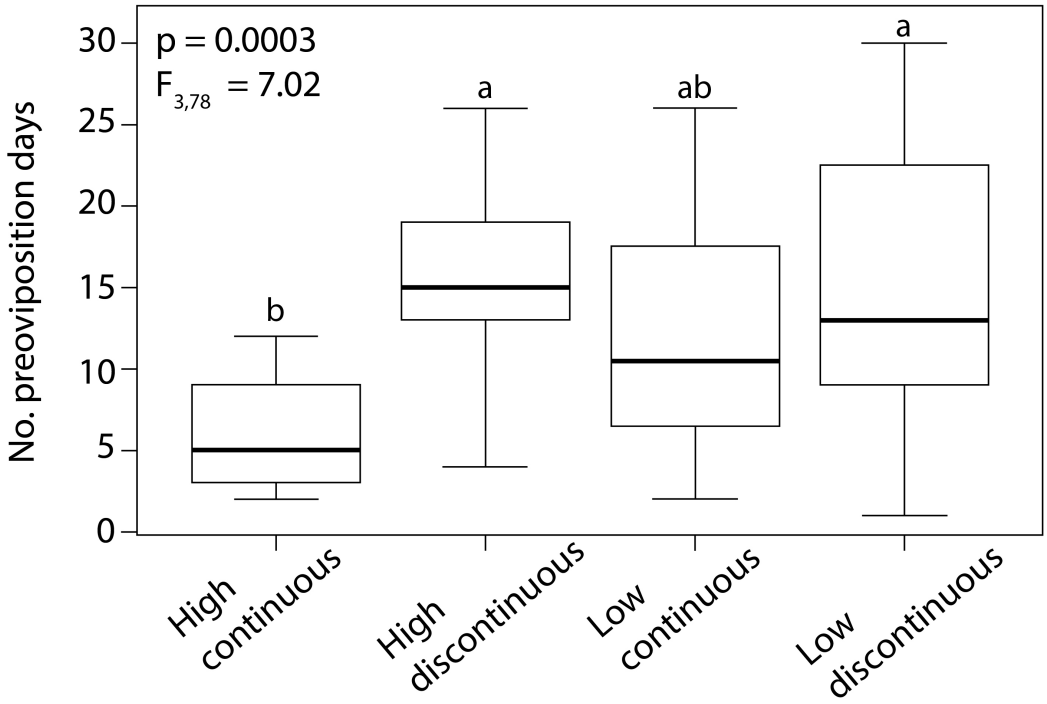

Figure 20 Median preoviposition periods, with interquartile range, of female *H. convergens* subjected to four different food supply treatments (High continuous = access to 6h of ad libitum food daily, High discontinuous = access to 12h of food every other day, Low continuous = access to 3h of ad libitum food daily, and Low discontinuous = access to 6h of food every other day). Treatments bearing the same letter were not significantly different (Tukey's test, $\alpha = 0.05$).

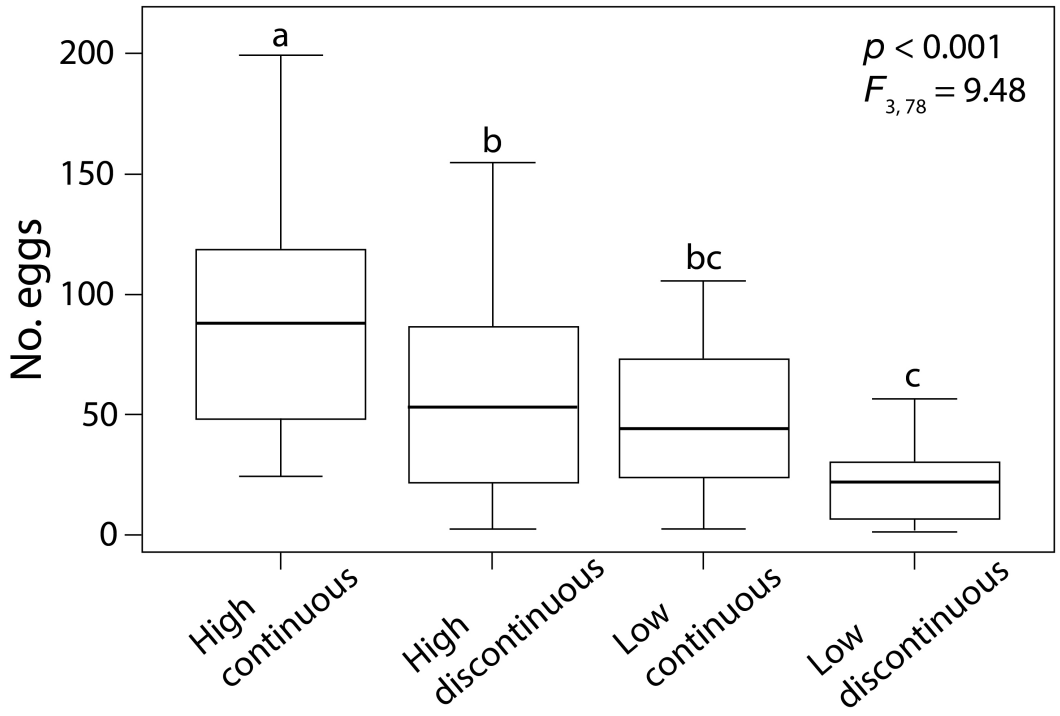

Figure 21 Median 18-d fecundities, with interquartile range, of female *H. convergens* subjected to four different food supply treatments (High continuous = access to 6h of ad libitum food daily, High discontinuous = access to 12h of food every other day, Low continuous = access to 3h of ad libitum food daily, and Low discontinuous = access to 6h of food every other day). Treatments bearing the same letter were not significantly different (Tukey's test, $\alpha = 0.05$).

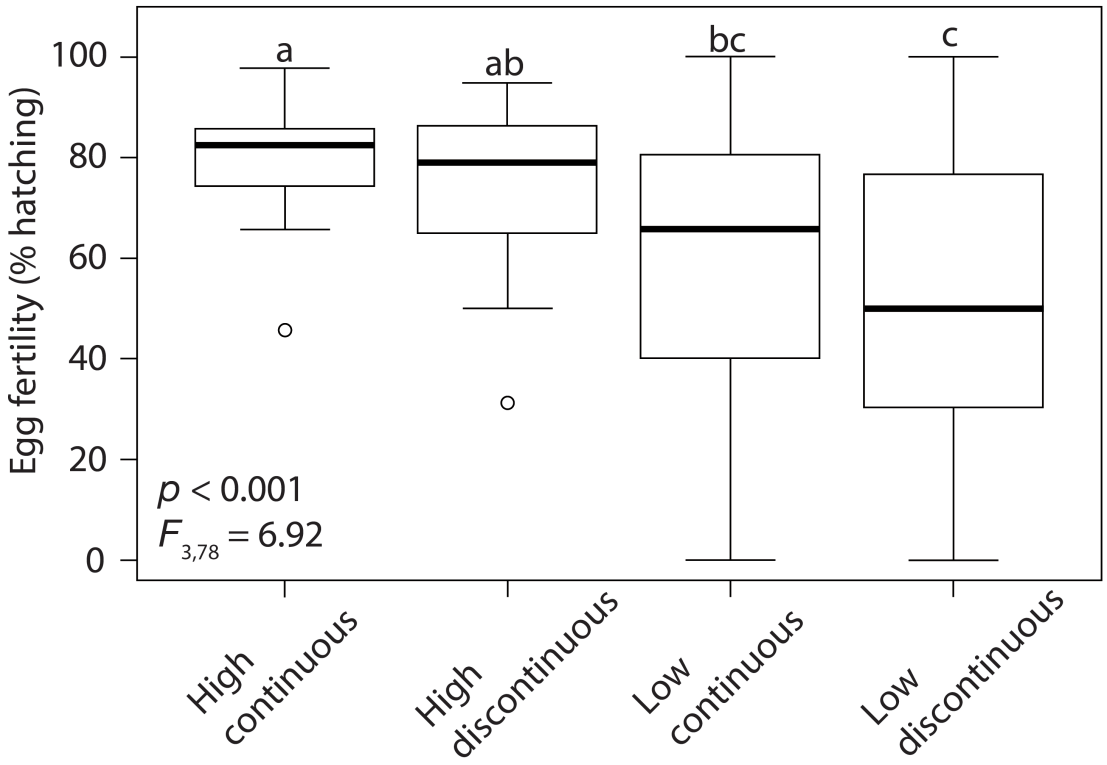

Figure 22 Median egg fertility, with interquartile range, of female *H. convergens* subjected to four different food supply treatments (High continuous = access to 6h of ad libitum food daily, High discontinuous = access to 12h of food every other day, Low continuous = access to 3h of ad libitum food daily, and Low discontinuous = access to 6h of food every other day). Treatments bearing the same letter were not significantly different (Tukey's test, α = 0.05).

Our best fit SEM (Figure 23) revealed the direct and indirect ways in which food amount and continuity affect reproduction and flight. We found that food amount ($\beta_D = 0.26$, $p = 0.03$) and pre-reproductive flight distance ($\beta_L = 0.32$, $p = 0.005$) both directly increased post-reproductive flight distance ($R^2 = 0.23$). On the other hand, continuity of food access influenced post-reproductive flight distance only indirectly via its effects on preoviposition period and number of oviposition days (path J -> N -> R). In particular, food access continuity decreased the

preovipositional period (β_J = -0.041, $p < 0.001$) which in turn decreased the number of oviposition days (β_N = -0.45, $p < 0.0001$). The number of oviposition days positively influenced 18-d fecundity (β_F = 0.79, $p < 0.0001$,and $r^2 = 0.81$, $p << 0.001$, Figure B) but was negatively correlated with total post-reproductive flight distance (β_R = -0.32, p 0.011 and $r^2 = 0.03$, p = 0.036 Figure 24) suggesting possible trade-offs between flight and fecundity.

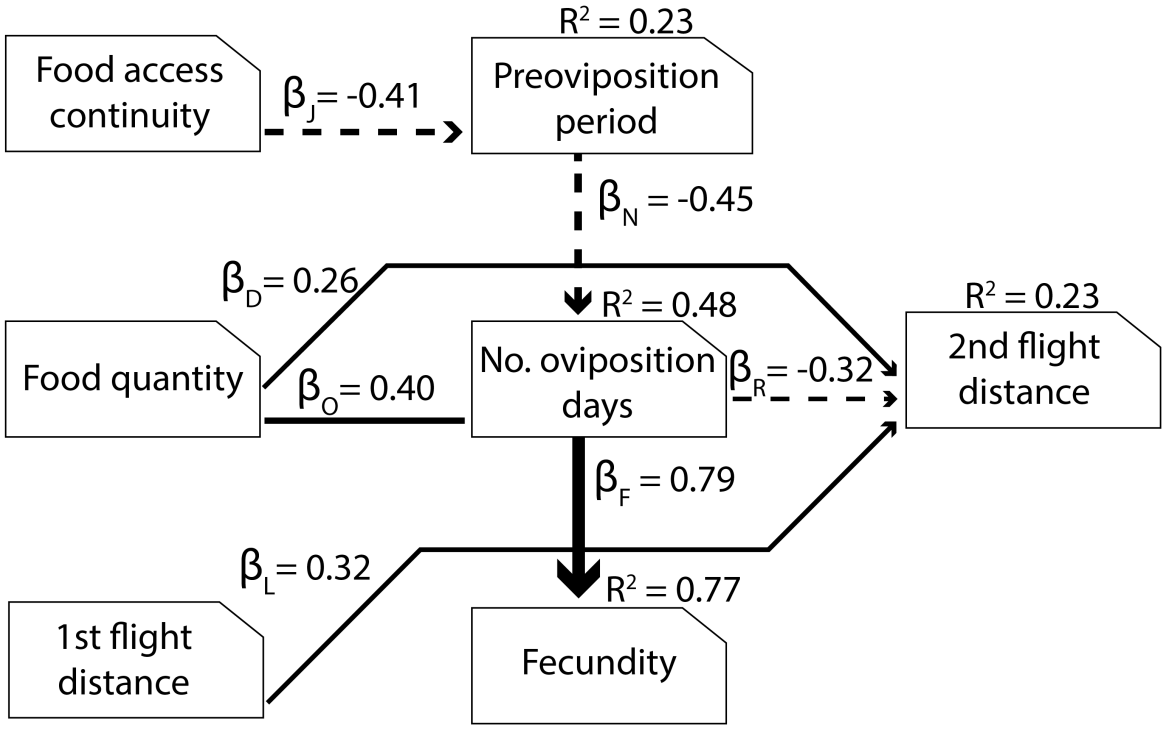

Figure 23 Structural equation model (SEM) of the effects of food treatments on flight performance and reproduction of female *H. convergens*. Solid arrows represent positive causal relationships and dashed arrows, negative causal relationships. Values are standardized path coefficients (β) with line sizes scaled to illustrate standardized coefficients. Coefficient of determinations (r^2) values indicate the proportion of the variation explained by the model. Fecundity is the number of eggs laid per female in 18 days of oviposition. Preoviposition period is the number of days from provision of the reproductive diet (greenbugs) to first oviposition. No. oviposition days is the number of days on which clutches were laid in the 18 day observation period. Flight distance is the total distance flown (in meters) during a 3 hour period on a tethered flight mill. Model fit: AIC = 53.8, df = 14, *p* = 0.31.

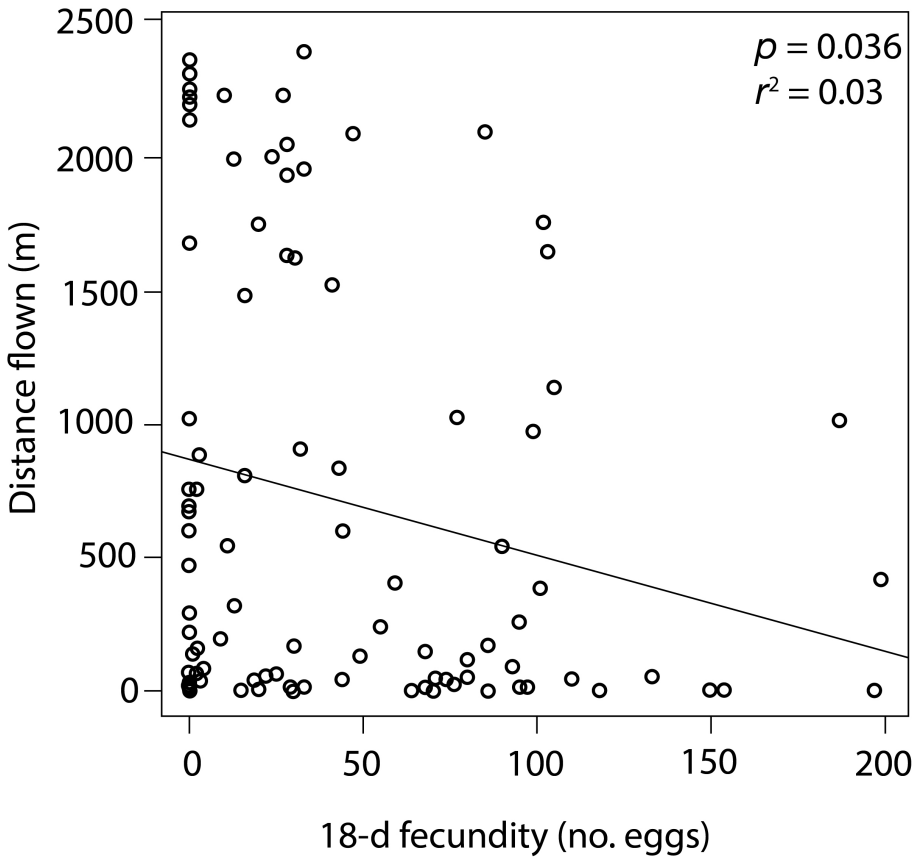

Figure 24 Linear regression of post-reproductive flight distances covered by *H. convergens* females on their 18-d fecundities. equation: y=-7.26x + 1744.2

4.5 | DISCUSSION

Both higher food quantity and higher continuity treatments influenced fitness through changing oviposition behavior, however the mechanisms by which this occurred varied. Higher food quantity increased oviposition days, whereas greater food continuity accelerated the onset of oviposition. These results support our initial hypothesis that both food access continuity and food quantity affect fitness metrics. Resource amounts appear to primarily affect fecundity, whereas continuity of resource access alters the timing of the onset of oviposition. Although the bottom-up effects of food resources, including plant-derived resources, have long been

recognized as an important influence for insect predators (Alomar & Weidenmann, 1996; Polis et al., 1998; Agrawal et al., 1999) most of this work has focused on resource quantity and quality (Rosenheim, 2001). By contrast, the influence of resource access continuity on insect predators has received much less study. Discontinuous access to dietary resources can thus influence predator's behavior though studies are rare. Resource continuity may potentially influence the community structure and population dynamics of insect predators in myriad ways and a fuller understanding of these avenues of influence will be critically important in the face of increasing environmental instability.

Egg fertility was decreased by lower food quantity treatments but was not affected by resource continuity. This result could indicate a higher rate of fertilization failure due to insufficient food, though varied resource quality often fails to affect fertility in *H. convergens* (e.g. Stowe et al., 2020). However, many Coccinellid species are known to lay mixed fertilized and trophic eggs in clutches (Michaud & Grant, 2004; Perry & Roitberg, 2005) and larval consumption of conspecific eggs increases developmental rate (Osawa, 1992, Michaud & Grant, 2004, Pervez et al., 2006, Omkar et al., 2007, Roy et al., 2007). Trophic eggs have been shown to increase in other coccinellids under lower food conditions (mainly *Harmonia axyridis;* Perry & Roitberg, 2005). *Hippodamia convergens* females engage in egg cannibalism behavior that benefits offspring life history traits and reduces future intraguild predation by consuming competitor clutches (Bayoumy & Michaud, 2015). Thus additional maternal behaviors that benefit larvae are plausible and exist in other coccinellids, though to our knowledge, larval provisioning through trophic eggs has not been investigated directly in this species. However, female *H. convergens* were observed returning to previous clutches and adding later eggs when the new, younger oviposition would be "destined for cannibalism by earlier hatching siblings"

(Bayoumy & Michaud, 2015). Future work exploring trophic provisioning in *H. convergens* is needed to determine whether fertilization is actively controlled in response to food quantity or if this is simply a potentially beneficial outcome of fertilization failure.

The onset of oviposition was delayed by more discontinuous resource access, especially in treatments with high food quantities. In fact, while the high resource quantity with continuous access beetles began laying eggs soonest, the high resource quantity with discontinuous access treatment delayed the longest in this experiment. Both the continuous and the discontinuous low food quantity treatment females began laying eggs sooner than the high, discontinuous treatment, illustrating that early oviposition is possible under discontinuous food access conditions. This suggests that the delayed oviposition we observed in the high, discontinuous treatment is not dictated by the diet conditions alone, but is rather, at least partially, a behavioral choice. Reproductive effects due to environmental variability, and resource discontinuity have been documented in insects (Johnson et al., 2016) and other animals (Millon et al., 2010, Nurul-Ain et al., 2017) though variability in resource continuity is indirectly studied through environmental effects on resources. Age at first reproduction can vary widely even within animal populations (Charmantier et al., 2006) and delaying the age of reproduction for a given population can slow population growth even without changing the total fecundity of individuals (Houston & McNamara, 1999; Millon et al., 2010). Reproduction also carries costs to the individual (Clutton-Brock, 1984; Viallefont et al., 1995), and delaying or reducing reproduction can extend survival in insects (Michaud & Qureshi, 2006). However, our results show that ovipositional delays indirectly reduce initial fitness through the reduction of oviposition days. All animals must balance current reproductive effort with the risk and potential rewards of later survival and reproduction (Williams, 1966; Roff, 1993; Stearns, 1992; Hadley et al., 2007). Under

discontinuous resource availability, our results suggest that the risk of early reproduction is sufficiently high to discourage reproductive effort, at least in the short term. Both resource amount and the continuity of resource access can influence fecundity; resource continuity, however, can have subtle but far reaching effects on oviposition behavior in insect predators.

Changes in natural enemy behavior patterns in response to energetic trade-offs may influence the pest control potential of a given predator through differential survival, reproduction or movement performance. Organisms often show reciprocal costs of expensive traits and behavior due to life-history theory (Roff, 1986; Zera & Denno, 1997; Zera & Harshman, 2001), which states that energetic 'capital' is preferentially routed to one of several fitness pursuits in response to environment, necessarily reducing energy for future output. However, research into energetic trade-offs concerning flight and fitness metrics have historically focused on energetic capital fueling greater investment into wing development in species with wing polymorphism (Harrison, 1980; Roff, 1986; Zera & Denno, 1997) rather than capital 'spent' on flight per se (Guerra & Pollack, 2009). Though flight behavior is complex and comprises multiple related metrics of bout length, distance, and flight propensity, here we use total flight distance to approximate metabolic cost in competition with reproduction to examine energetic trade-offs. In this study, we showed that increasing reproductive days, instead of fecundity, decreased post-reproductive flight distance while initial flight distance was correlated with overall lower fecundity in a direct comparison. These results are in line with life-history theory, however higher initial pre-reproductive flight and higher post-reproductive flight distance were positively correlated, contradicting our hypothesis that greater initial flight distance would decrease later flight distance. Flight capacity in insects often varies with age, condition, and physical damage to the wing or the insect body (Carey et al., 2006; Miller et al., 2008; Vance, 2009) however due to

the controlled nature of this experiment, it is unlikely that individuals were varied in condition or damage and were identical ages. Rather, the variation in initial flight more likely reflects intrinsic individual differences in innate flight distance capacity in *H. convergens*. It is also possible that the initial flight period was insufficiently challenging to reveal any negative effect on a second flight. Future research could increase the flight testing period or allow flight repeatedly to ensure significantly burdensome energetic movement cost. Greater distances between resource patches or more frequent moves increases the metabolic output necessary for survival and likely reduces the energy devoted to future egg production or flights in insects (Schmid-Hempel et al., 1985; Guerra & Pollack, 2009). Movement between patches also represents significant predation, exhaustion, and physical damage risk along with simple starvation in the matrix (Bonte et al., 2012). Though movement by flight in response to resource scarcity or patch depletion is clearly necessary in agricultural systems to avoid starvation, It is critical that we understand the complex environmental cues that influence behavioral trade-offs between flight and fitness to effectively support mobile insect predators and the ecosystem services they provide.

Agricultural systems experience high levels of seasonal predator and pest resource variability due to harvest cycles and other human disturbances. Conservation practices have primarily focused on increasing resource amount and diversity to support ecosystem services in the past (Power, 2010). More recently however, resource access variability has begun to gain attention as a potential target to support ecosystem services (Schellhorn et al., 2015; Angeler et al., 2016; Kennedy et al., 2016; Cohen et al., 2017; Cateau et al., 2018; Egli et al., 2020; Iuliano & Gratton, 2020; Spiesman et al., 2020). Ensuring a more even distribution of resources in space and time on the landscape, as a complement to current efforts to increase resource amount and diversity, could benefit insect populations without requiring prohibitively higher absolute inputs.

More continuous availability of food resources has been shown to decrease insect emigrational behavior in planthoppers (Denno et al., 1991), and our results here show that more continuous feeding led to earlier oviposition and through this effect, more oviposition days (path n). Increasing resource continuity through the judicial application of cover-crops, management of non-crop habitat, or cash crop arrangement on the landscape may materially improve the predation services of natural enemies in agricultural fields (Landis et al., 2000; Tscharntke et al., 2005; Bianchi et al., 2006; Rusch, 2010; Rayl et al., 2018), ensuring support for generalist insect predators without substantially increasing the absolute quantities of resources provided in a given patch each year.

This study illustrates how the interactive and complex nature of resource access (i.e. amount and access variability) may alter various aspects of *H. convergens* behavior and fitness. Teasing apart resource amount and continuity effects illustrate complex behavioral and fitness tradeoffs between different aspects of resource needs in these important predatory insects. Identifying avenues of fitness influence provides several targets for valuable ecosystem support, as resource availability can alter reproduction in both amount and timing. Understanding the way environmental cues alter natural enemy responses can improve our ability to shape management strategies with care and nuance. Biological control agents benefit from increased resource availability, and as biological control services become increasingly crucial to ecosystem services, we can increase this benefit by considering resource availability in time in addition to availability in space. Conserving native populations and supporting their ecosystem services provides control of pest species that threaten row crops in agricultural systems around the world. Improving resource continuity on the landscape must be considered in concert with resource amount to maximize the survival and population growth of generalist insect predators like *H. convergens*.

4.6 | REFERENCES

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Chapter 5 - General Discussion

5.1 | DISCUSSION

The results presented in this thesis pertain to several connected questions that ultimately relate to the performance of the predatory lady beetle *H. convergens* in the context of biological control. Agricultural fields often comprise vast monocultures that reduce the diversity of resources available to beneficial insects. Understanding the impact of diet diversity in *H. convergens* lady beetles can help to identify the role of resource diversity in effective predation ecosystem services. Pursuant to this goal, I first examined the developmental and reproductive effects of diet diversity on larval *H. convergens* lady beetles. I then investigated adult *H. convergens* reproductive and flight responses to the omnivorous vs prey-only diets and adult beetle size to further explore the importance of diet diversity, and particularly the importance of plant-derived resources. Plant-derived resources often differ from animal derived resources in macronutrient profiles, nitrogen content, and other trace nutrient composition. Thus, diverse diets that include plant-derived elements can improve the performance of insect predators more than diets that are diverse with respect to prey components alone. Finally, I examined the reproductive and flight performance of *H. convergens* in response to resource quantity and continuity. Due to harvesting cycles and seasonal temperature and precipitation variability, cropping systems can undergo spatial and temporal shifts, creating a mosaic of ephemeral resource patches on a seasonal scale and necessitating individual and population movement to access distant resources.

Altogether the results presented here show the critical importance of resource type and arrangement on the landscape. The resources encountered by insect predators can influence their performance in myriad ways and both identifying these avenues of influence and understanding

their potential effects will be crucially important to the continued success of conservation biological control. Land management practices focused on improving the resource landscape for insect predators must take these diverse influences into account to provide maximum benefits to the efficacy of ecosystem services in the agroecosystem especially. Maximizing benefit 'rate-of-return' from land management efforts is especially important in the agroecosystem since land is highly sought after for crop production. Identifying and providing the best possible resource types and spatial and temporal arrangement can provide maximum support for minimum investment inputs in grower effort, land use, and cost.

5.2 | Chapter 2 – The Benefits of Omnivory for Reproductive and Life History in a Specialized Aphid Predator, *Hippodamia convergens* (Coleoptera: Coccinellidae)

The results reported in Chapter 2 of this thesis indicated how diet diversity in the larval stage can alter developmental rate and adult body size. From a purely developmental perspective, a non-diverse, aphid-only diet appears to be beneficial, slightly speeding development and increasing fresh weight at emergence for adults. This was a surprising result as we had expected higher quality (i.e. more diverse) diets to increase developmental rate and adult body size rather than decrease it relative to lower quality (i.e. less diverse) diets. However, protein-rich diets are sometime preferred by insect predators under resource depleted conditions (Raubenhiemer and Jones 2006, Raubenhiemer et al. 2007) and protein seems to be the specific macronutrient driving prey selection behaviors in many insect predators (Jensen et al 2012, Mayntz et al. 2005). Indeed, protein heavy diets have been shown to speed coccinellid development before (de Clercq et al. 2005, Jalali et al. 2009, Hodek and Evans 2012). Therefore, I expect that the higher

concentration of protein in the prey-only diet relative to the diverse diet, may be responsible for the increased developmental rate observed in these results.

In spite of the early benefit of a non-diverse diet to developmental rate, when the monotypic diet is continued throughout adult life stages oviposition days are reduced, resulting in lower overall fecundity. By contrast, adults that had access to a diverse diet showed higher fecundity than those eating a monotypic diet, regardless of the diversity of their larval diets. This indicates that adult diet supplementation with diverse components can potentially compensate for the ill effects of a larval diet with restricted compositional diversity. In spite of the relatively large difference in adult fecundity between diet treatments, we observed no difference in the fertility of the eggs laid. It seems therefore, that the reproductive benefits of diet diversity may outweigh the slight developmental delay cost in the larval stage.

5.3 | Chapter 3 – Plant Resources Mediate Reproduction and Flight in a Specialized Aphid Predator, *Hippodamia convergens* (Coleoptera: Coccinellidae).

My questions in Chapter 3 build upon the results of Chapter 2 and focus specifically on the role of plant resources as a diet component rather than diversity itself irrespective of component type. Additionally, since results indicated that adult diet was more critical to reproductive performance in *H. convergens*, I focused my research objectives mostly on adult diet composition. I manipulated diet quantity during larval development to create adult size classes that stand in for high and low quality natal patches, instead of investigating direct effects of larval diet. Additionally, flight assays were conducted to test the influences of diet on energetically intensive behavior and to provide a competing energetic cost to reproduction. I expected trade-offs to exist between these two fitness critical behaviors and hypothesized that

larger body size and omnivorous diets would be beneficial in both treatments though perhaps to different degrees.

I found that large adult body size increased ovipositional delay, but decreased number of oviposition days, while only marginally decreasing individual fecundity. These findings were counter to my expectations, and I believe that additional work may be warranted to understand the effect of adult body size on reproductive metrics, as it stands this appears to suggest that larger insects have lower fitness than smaller insects in this species. One reason for this odd result may be that larger body size allows females to wait longer to begin oviposition due to a greater volume of 'storage' space in the abdomen. A study that tracks the lifelong fitness of adults that vary in size would be instructive in determining the rectitude of this possibility. However, it is important to note that regardless of lifelong reproductive capacity, delaying early reproduction is a risky strategy as mortality threats are always present in the field and any insect delaying reproduction runs the risk of dying prior to passing on its genes. However, the odd reproductive effect of larger body size could also be an artifact of the *ad libitum* adult treatments, as in natural contexts insects would experience greater resource restriction than our insects faced. Thus our large insects may have achieved a size significantly larger than possible in the field. If this is the case, effects of this unnaturally large body size may not be adaptive. Additional work is necessary to determine whether large adult body size in more natural, resource restricted, environments would have a similar effect.

Omnivorous diets were beneficial to *H. convergens* reproduction, shortening preoviposition period, increasing the number of oviposition days, and increasing individual 18-d fecundity. This supported my hypotheses, indicating that a relatively small proportion of bee pollen and diluted honey can increase reproductive performance even relative to an *ad libitum*

diet of greenbug aphids and frozen *E. kuehniella* moth. As we seek to support beneficial insect predators these results indicate that our focus must not only be providing sufficient prey quantity and quality but also in providing diverse plant resources.

In spite of these clear results of diet and size on reproduction, flight capacity results were more complicated. Neither treatment factor affected flight distance in either of the individual flight assays or in the cumulative total distance traveled in both testing periods. However, we did see that reproductive metrics such as longer preoviposition period increased post-reproductive flight distance. Given the lack of influence from early flight to later reproductive metrics, it is possible that this indicates a response to 'patch' conditions (i.e. resource composition and amount). In other words, the correlation between oviposition delay and greater dispersal effort, could indicate that conditions that cause females to delay reproduction also encourage increased dispersal distances.

The relatively fine differentiations in flight distance between treatments in this experiment however indicate that if there is indeed such a relationship, either greater flight challenges or greater resource restriction is necessary to reveal it. Published work on *H. convergens* flight was limited and the only estimate of flight duration found (Jeffries et al 2020) stated flight times "up to two hours" with averages of 36.5 minutes in this species. Our observed flight distances seem to suggest a greater flight capacity than these published values, though this could be due to differences in the methods of rearing and flight measurement or in the health and condition of the individuals measured. It is also plausible that the flight period insects in this experiment were allowed was simply insufficiently close to the true flight capacity of these insects to present a substantial energetic challenge. However, it is also entirely possible that such

long flights are not common in nature and thus the trade-offs between reproduction and flight in *H. convergens* are not ecologically meaningful except in extreme circumstances.

5.4 | Chapter 4 – Resource amount and Access Period Influences Flight and Reproductive Behavior in *Hippodamia convergens* (Coleoptera: Coccinellidae).

The diet of *H. convergens* is not depended on resource composition alone but also on the timing of resources availability. If every field within flight range on the landscape is flowering at the same time, the total amount of resources available for the year may appear to be sufficient to support a robust community of insect predators. However, the synchronized availability may lead to a concentration of resources in one particular time period, leaving the rest of the year with relatively few resources. In that situation then, the temporal dynamics of resources availability may be insufficient to support a robust population even though the absolute quantity of resources may appear sufficient. In other words, resource amount may actually obfuscate the otherwise cryptic periods of prey scarcity that may prevent predator establishment. This issue is becoming more recognized in the literature (Welch and Harwood 2014, Schellhorn et al. 2015, Spiesman et al. 2020) and my aim with this was to examine how short-term resource access discontinuity influenced ecologically important performance metrics of an omnivorous insect predator.

In Chapter 4 of this thesis we were interested specifically in how the continuity or discontinuity of resource access would alter reproductive and flight performance of *H. convergens* adults. However, since varying continuity of resource access necessitates varying amount as well, I applied high and low food access conditions to disentangle these effects. This also allowed for an investigation into how food amount altered reproduction and flight capacity at the same time.

Discontinuous resource availability altered reproduction through extended oviposition delay. This supported my hypothesis and suggested that resource continuity is important to the ecological performance of insect predators even when total resource amount on the landscape is sufficient. Though resource amount is still critical for survival of beneficial insects, reproductive delays and lower early fecundity can still significantly alter the population dynamics of a given predator population. Such a delay could also decrease biological control potential long term as slower predator growth rates allow pest populations peaks to increase before being brought under control, increasing the probability of pest adaptations (evolutionary rescue, Michaud 2018). Thus even though overall fecundity was not affected as much by resource access continuity as by resource amount, such effects can still represent extensive ecological alterations to the success of predatory insects in the agricultural ecosystem.

Interestingly, and in contrast to both Chapter 2 and Chapter 3 results, we did see a difference in fertility in this experiment. Since conditions are different between experiments, we cannot make direct comparisons, however the fact that out of all the conditions applied to *H. convergens* lady beetles only total resource amount appeared to significantly alter fertility suggests some additional interesting questions. Trophic eggs have been documented in predatory coccinellids in prior work (Michaud & Grant, 2004; Perry & Roitberg, 2005) and it is possible that this dip is fertility is due to maternally controlled decrease in fertilization to provision larvae with early meals in the form of 1st instar cannibalism of infertile eggs in their clutch. However, this fertility decrease could also be the results of simple failure of maternally controlled fertilization due to insufficient resource availability during the maturation and oviposition of the eggs. The experiments conducted here are not designed to explore this question so additional

work would be necessary to fully reveal any influence between diet amount and fertilization that may exist.

Structural equation modeling in this experiment also revealed interesting flight results and life history trade-offs between reproduction and flight. In Chapter 3 we also examined the flight behavior of this species but found very few effects of diet continuity and timing, while diet amount was shown to have relatively strong direct and indirect effects on later flight distance capacity, with greater resource amount availability increasing later flight performance.

5.5 | General Conclusions

The most minimal form of conservation biological control is simple passive avoidance of harmful management strategies that decrease the population sizes, efficacy, and habitat for natural enemies (Rusch et al. 2017). However, in the agroecosystem, this simple avoidance of harm is insufficient due to the prior tillage, pesticide applications, decreased shelter habitats and other similar human generated landscape alteration (Rusch et al. 2017). In other words, agricultural landscape carry a long tradition of harmful management strategies that create a foundational barrier to biological control of pests. The current, wide-spread, industrialized approach selectively supports pest populations due to vast monocultures of synchronized resource booms, and the relative dearth of remnant natural areas (Letourneau et al 2011). In this landscape, natural enemies can struggle to persist *in situ* due to human management such as frequent mowing, harvest cycles, and chemical pesticide applications. Thus, for conservation biological control approaches to thrive management practices must seek to provide the maximum possible benefit in the minimal possible area (Popp et al. 2013). In pursuit of this goal, it is

increasingly important that land management strategies identify additional avenues of influences and tailor support to provide benefits in as many ways as possible.

Population dynamics in the agroecosystem are integral to the efficacy of conservation biological control. Understanding details about an organisms biology and ecology is important to design and provide better support their population survival, growth rate, and movement ability about the landscape (Rusch et al. 2017). My results here show that increasing diversity of the resource landscape, especially in plant derived resources, leads to greater fecundity and earlier oviposition in *H. convergens*, both important factors to the population growth rate. Increasing the population growth rate can provide faster control of pest outbreaks and ultimately decrease the number of outbreaks that reach economic threshold (Bianchi et al. 2006). This information therefore can be used to improve the efficacy of ecosystems service support from natural areas and improve our management strategies through an emphasis on the important of plant resources for insects predators. My results here also indicate the importance of greater continuity of resources in the landscape. Greater continuity of resources led to earlier oviposition and higher fecundity. Additionally, understanding movement can be critical to conservation biological control in agroecosystems as natural enemies must often seek resources outside the crop production field itself (Ekbom 2000). The flight distances measured here illustrate the incredible flight capacity H. convergens can achieve, however, low resource amounts can decrease flight distance. Finally, I found some evidence of energetic trade-offs between reproduction and flight. Reproductive effort may decrease future flight, altering the movement capacity of *H. convergens* on the landscape and decreasing reproductive individual's ability to access resource patches outside of the crop field, and reducing their access to diverse resources. According to these results an idealized resource patch for *H. convergens* would include diverse prey and plant

resources and would provide these resources continually throughout the year and distributed about the landscape in such a way that insects foraging for pests in crop fields can reach them without traversing exhaustive distances.

In general, results found here indicate the importance of both greater diet diversity of plant and prey components, along with greater resource continuity on the landscape. In addition to applying cover crops around crop fields, integrating diverse resources into the landscape through intercropping strategies, or simply crops in complementary schedules that ensure resource booms occur at different times throughout the year could increase both the diversity and continuity of resources in the High Plains agroecosystem. These increases, in turn, could increase the populations sizes of beneficial insect predators such as *H. convergens* along with increasing their timing of reproduction and growth rate after pest outbreaks (Bianchi et al 2006, Chaplin-Kramer et al 2011). Flight behavior effects of diet diversity and composition are harder to untangle, however additional research into the flight performance of these important natural enemies would be invaluable in understanding how these beetles move about the landscape and the costs they bear due to the fragmentation and disturbance of industrialized agricultural practices.

In this thesis, I explored several categories of resource access, composition, continuity, and amount for one species of generalist insect predator, *H. convergens*. Examining these categories together can inform our land management strategies to better support this species, and thus, its ecosystem services in the agroecosystem. However, similar examinations of other species that provide predation services are equally important. The most beneficial support practices for *H. convergens* may not be ideal for other generalist predators or even other Coccinellid predators. In spite of this individuality of needs, there are likely important general

improvements that can increase overall ecosystem support in a landscape, even though some individual species may be disadvantaged (Bianchi, Booij, and Tscharntke 2006). Resource diversity and continuity appear to be among these generally beneficial management improvements and their improvement in the agroecosystem could materially benefit predation ecosystem services and ultimately crop protection from damaging pests.

5.6 | References

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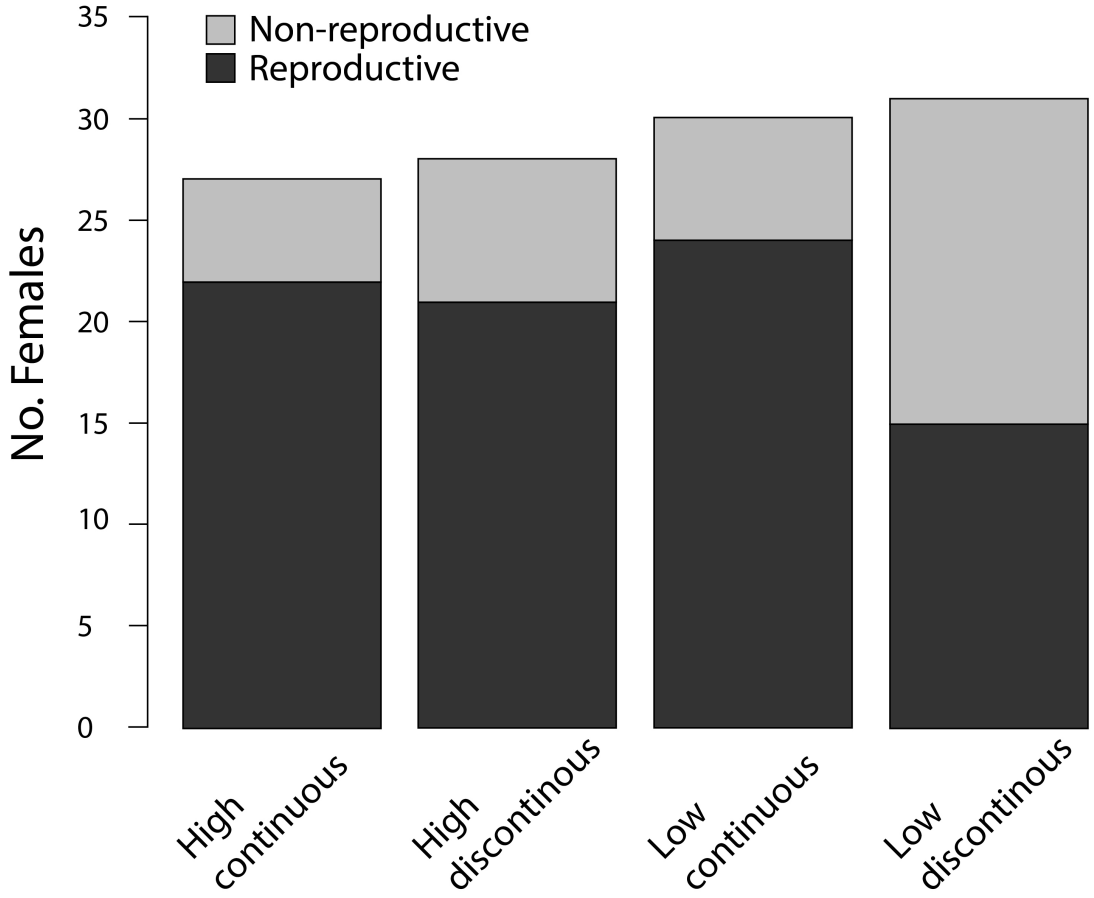
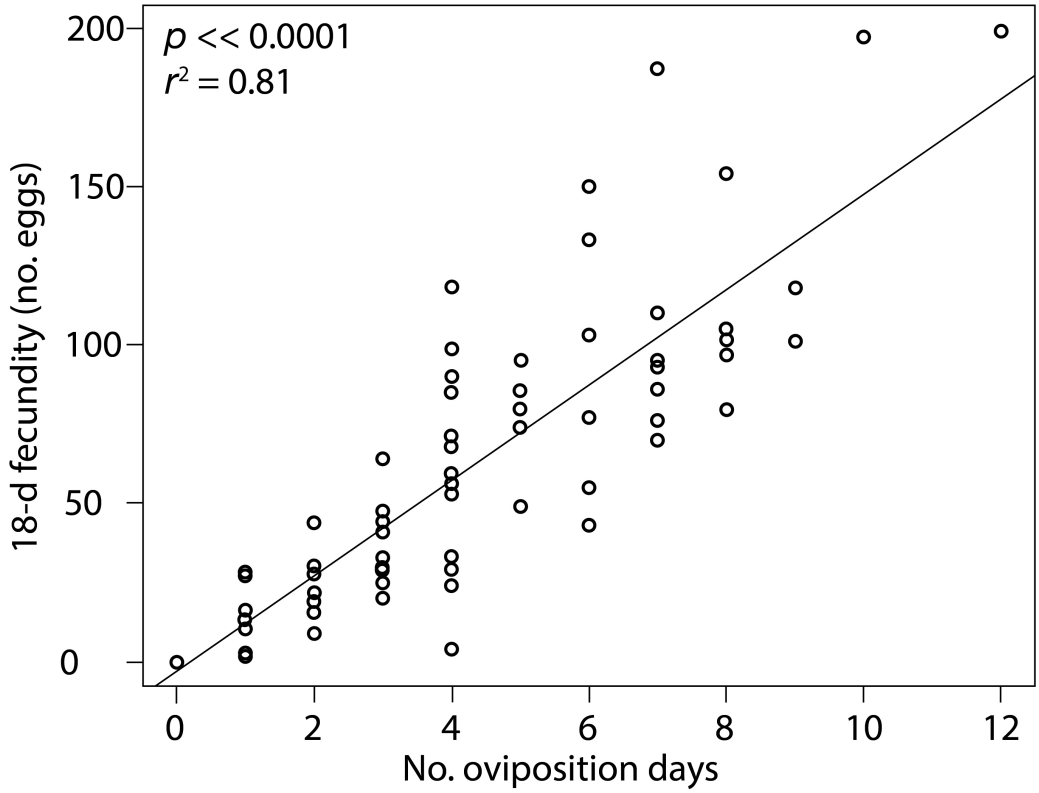
Reference lists appended to end of the relevant chapter.

Appendix A - Chapter 4 Appendix



Appendix A Number of Female H. convergens females breaking diapause in four different food supply treatments (High continuous = access to 6h of ad libitum food daily, High discontinuous = access to 12h of food every other day, Low continuous = access to 3h of ad libitum food daily, and Low discontinuous = access to 6h of food every other day). X^2 = 10.47, df = 3, p = 0.015,



Appendix B Linear regression of individual 18-d fecundities by *H. convergens* females on their number of oviposition days. equation: y=15.02x – 2.61